Vol. 52, No. 7, December, 1961
Russian Original Dated July, 1961

BULLETIN OF EXPERIMENTAL BIOLOGY AND MEDICINE

БЮЛЛЕТЕНЬ ЭКСПЕРИМЕНТАЛЬНОЙ БИОЛОГИИ И МЕДИЦИНЫ

BYULLETEN' ÉKSPERIMENTAL'NOI BIOLOGII I MEDITSINY

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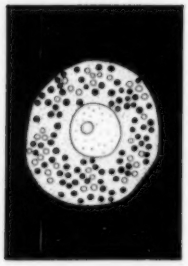

ACHIEVEMENTS IN SOVIET MICROBIOLOGY

Edited by A. A. Imshenetskii

Director of the Institute of Microbiology
of the Academy of Sciences of the USSR

TRANSLATED FROM RUSSIAN

(Original published for the Institute of Microbiology, Academy of Sciences of the USSR, by the Academy of Sciences Press, Moscow)

This concise survey of recent developments in (as well as projected directions of) Soviet microbiology is divided into four major sections:

I. Progress in General Microbiology, *A. A. Imshenetskii*
A broad review which enables the Western scientist to see clearly the direction in which Soviet microbiologists are making their studies.

II. Achievements of Soviet Microbiology in the Field of the Study of the Geological Activity of Microorganisms, *S. I. Kuznetsov*

In a field which has not been widely considered by Western scientists, this paper indicates great advances made in the USSR. The use of microorganisms in petroleum research and recovery are discussed, as are Soviet efforts to classify precisely and map out geologic or ecologic microbiological strata as they occur in the geologist's scheme. This article summarizes Soviet research into the relationships of various groups of microorganisms to geological – petroleum – inorganic substrates environments.

III. Forty Years of Technical Microbiology in the USSR, *V. N. Shaposhnikov, N. D. Ierusalimskii, and I. L. Rabotnova*

Noteworthy in this paper (which deals with fermentation microbiology, acetone-butanol, organic acids, riboflavin, antibiotics, etc.) is Shaposhnikov's description of disagreement in the USSR, as there has been in the West, on the biosynthesis of citric acid by various species of *Aspergillis*.

IV. Achievements in the Field of Soil Microbiology in the USSR, *E. N. Mishustin*

Soviet research in this area places particular emphasis on rhizosphere, root nodule, and nitrogen-fixing bacteria, and on the relationship between higher plants and associated microorganisms. Special attention is given to Vinogradskii's early description of auxotrophs and Krasilnikov's recent classification of actinomycetous microorganisms. Some comment is made on methodology, especially microbial counts and selection techniques.

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BULLETIN OF

EXPERIMENTAL BIOLOGY AND MEDICINE

A translation of BYULLETEN' EKSPERIMENTAL'NOI BIOLOGII I MEDITSINY, a publication of the Academy of Medical Sciences of the USSR

(Russian Original Dated July, 1961)

Vol. 52, No. 7, pp. 747-868 December, 1961

CONTENTS

	PAGE	RUSS. PAGE
PHYSIOLOGY		
The Effect of Hyaluronidase Inhibitors on Urinary Excretion. A. G. Ginetsinskii and V. F. Vasil'eva	747	3
The Reflex Pathways of the Anticoagulation System. T. M. Kalishevskaya, B. I. Kotlyar, and B. A. Kudryashov	750	5
The Effect on Cardiac Reflexes of Removal of the Chromaffin Tissue. Z. V. Urazaeva	754	9
The Influence of Urea on Nervous Stimulation of Cardiac Muscle. A. A. Mazurok	759	15
Adaptation of the Visual Apparatus to Natural Illumination. A. P. Anisimova	762	18
Measurements of the Elasticity of the Mammary Gland as Related to the Human Milk Ejection Reflex. M. G. Zaks and I. A. Mazhbits	765	20
Insulin Secretion by the Pancreas of the Chick Embryo. L. G. Leibson, Z. P. Zheludkova, and L. I. Chilingaryan	768	24
The Effect of Sham Feeding on the Feeding Behavior of Esophagotomized Dogs. V. D. Smirnova	772	28
PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY		
Change in the Sorptive Properties of a Myocardium Affected by Adrenalin. Z. I. Vedeneeva	776	33
Distribution in the Organs and Tissues of Intravenously Administered Radioactive Phosphorus in Infectious Peritonitis. E. M. Neiko	781	38
The Effect of Benzene on the Leuko-Agglutinating and Reticulocytogenic Properties of Sera. A. Ya. Yaroshevskii	784	40
The Influence of the Nervous System on Bone Marrow Hemopoiesis. M. R. Rakhmatova	788	45
Changes in Body Temperature and Oxygen Consumption in Rabbits Following the Administration of Sodium Amytal. G. M. Daudova	792	48
The Role of Ascorbic Acid in the Mechanism of Artificial Hypothermia Development. K. V. Ostashkov	797	54
Effects of Some Nucleic Acid Components and of Their Precursors on Incorporation of [1-C^{14}] Glycine and [1-C^{14}] Tyrosine into the Proteins of Normal and Tumor Tissue. S. Ya. Davydova	801	58
Cholesterol Metabolism, Acetylation Processes, and Thiamine. Yu. M. Ostrovskii	805	62
The Glycogen Content in the Liver and Muscles of Rats in Relation to the Time Elapsed after Administration of Chloropropamide and to the Duration of Fasting. S. G. Genes, L. M. Makarevich-Gal'perin, and S. N. Ushenko	808	65
The Effect of Ultrahigh-Frequency Currents on Blood Serum Protein Fractions. R. G. Singatullina	812	69

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PHARMACOLOGY

Hyperkinesis of Central Origin Induced by Tetridine and Its Inhibition by Anticholinesterase (Choline-Sensitizing) Agents. N. V. Uskova 816 73

Inhibition of Glutamic-Pyruvic Transaminase by DL-Cycloserine and Other Compounds. E. D. Vyshepan, K. I. Ivanova, and A. M. Chernukh 819 76

MICROBIOLOGY AND IMMUNOLOGY

On the Immunological Characteristics of Serum Proteins. Communication II. Determination of the Content of Protein Fractions in the Globulin Composition Immunologically Similar to Albumins. Yu. S. Tatarinov 823 81

ONCOLOGY

On the Specific Antigen of Human Sarcomas. V. S. Korosteleva 826 84

The Role of the Lymph Nodes in Acquired Antitumor Immunity. Communication I. Changes in the Regional Lymph Nodes Following Homotransplantation of the Brown-Pearce Tumor. A. F. Zakharov 831 89

The Influence of Hibernation on the Development of Experimental Tumors in Citellus Maximus Pall. Z. Ya. Belogrudova 837 96

EXPERIMENTAL BIOLOGY

Ways of Transport of the Secretion from the Hypothalamic Centers into the Neurohypophysis. A. A. Voitkevich 840 99

The Daily Periodicity of the Mitotic Activity in the Corneal Epithelium of Starving Rats and Mice. O. T. Movchan 845 103

The Influence of Bacterial Polysaccharides upon Experimental Tissue Regeneration. A. I. Braude, G. E. Vaisberg, T. I. Afanas'eva, and N. I. Givental' 849 107

Peculiarities in the Regeneration of the Hematopoietic Tissue in Chick Embryos Exposed to External γ-Radiation. A. V. Bursian 852 110

MORPHOLOGY AND PATHOMORPHOLOGY

The Blood Indices of the Healthy Guinea Pig. E. D. Gol'dberg..... 856 115

METHODS

A New Method for Producing an Isolated Gastric Pouch. E. I. Rozova 859 119

Testing Small Insulin Concentrations by Means of Isolated Epididymal Rat Fat. L. L. Liberman 861 121

The National Institutes of Health of the Public Health Service has supported in part the cost of publishing BULLETIN OF EXPERIMENTAL BIOLOGY AND MEDICINE through the purchase of copies for distribution to American medical libraries.

THE EFFECT OF HYALURONIDASE INHIBITORS ON URINARY EXCRETION

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(Presented by Active Member AMN SSSR V. V. Parin)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 52, No. 7, pp. 3-5, July, 1961
Original article submitted July 19, 1960

Recently, our laboratory has issued reports [1,2,3,4,5] which have shown that the facultative reabsorption of water which has been excreted from the distal part of a nephron under the influence of the antidiuretic hormone is due to the action of hyaluronidase. Through the depolymerization of hyaluronic acid, which is a component of the intercellular cement of the nephron's wall, the cement is made permeable to water, which is then reabsorbed osmotically.

If this hypothesis is correct, substances which inactivate hyaluronidase would be expected to restore the impermeability of the walls of the tubules to water, and so prevent facultative reabsorption. Antihyaluronidases should therefore increase diuresis.

The present work has been undertaken to test the correctness of this conclusion, which follows from our hypothesis on the mode of action of the antidiuretic hormone.

METHOD

The work was done on white rats. In one set of experiments, a fistula was established in the urinary bladder which allowed urine to be collected continuously. In another set, four rats were placed in a cage having a base made of wire mesh, and the urine was collected through a funnel placed beneath it. In these experiments, a record was made of the amount of urine excreted over a period of several hours when the excretion was voluntary. As hyaluronidase inhibitors we used heparin and ascorbic acid. The antihyaluronidases were injected intraperitoneally. The ascorbic acid was first neutralized with sodium bicarbonate, and was given as a dose of 0.1 g per 100 g weight. The amount of heparin injected was 1000 units per 100 g weight. The concentration of sodium in the urine was measured by flame photometry and by the inulin-resorcin method.

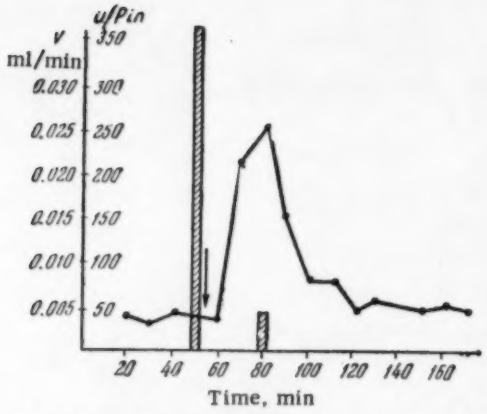
RESULTS

The rats were injected with inulin before the experiment. After a few control portions of the urine had been collected, the ascorbic acid was injected intraperitoneally. Immediately after the injection, the diuresis rose from 0.005 to 0.025 ml/minute – a fivefold increase. When the effect of the single injection of the antihyaluronidase agent had worn off, urinary excretion returned to normal.

The results of a typical experiment are given in the figure.

Measurement of the inulin concentration ratio shows that the polyuria produced by the ascorbic acid is caused by a marked reduction in facultative reabsorption. In the control period, the ratio of concentration of inulin in the urine and plasma exceeded 350, which represented a condition of oliguria. After the ascorbic acid had been injected, the ratio was reduced more than seven times, indicating a great reduction in the reabsorption of water, despite the fact that the rat still has a water deficit. A similar effect was induced by injecting heparin.

In the second set of experiments, 1 ml of 10% ascorbic acid per 200 g weight was injected hourly for five hours. The results obtained are shown in the table, which gives the mean values of all the determinations.



The effect of ascorbic acid on the excretion of urine in a rat with a urinary fistula. The columns indicate the concentration index of inulin. Ordinate-diuresis in ml/minute (V) and the ratio of the inulin concentration in the urine to that in the plasma (u/P index); abscissa-time in min. Time of injection of ascorbic acid shown by arrow.

Before testing the action of hyaluronidase inhibitors, a control determination was first made in all the animals. All the results concerning the excretion of water and sodium were obtained under standard conditions of moderate water deficiency, induced by keeping the animals without food or drink for 18 hours.

Ascorbic acid injected into the same animals and under the same experimental conditions increased urinary excretion five times. At the same time there was a marked increase in the amount of sodium excreted, and the concentration in the urine did not fall, as it would if the increased diuresis were the only effect, but it actually rose. In order to eliminate any possible error due to the fact that some sodium would be introduced together with the ascorbic acid, a special control experiment was arranged. As would be expected, injecting sodium bicarbonate in the same amount as was required for neutralization caused only a very small and statistically insignificant increase in urinary excretion. At the same time sodium excretion was increased 60%, whereas after the injection of ascorbic acid, the amount of this ion excreted increased eight times.

Results of the Second Set of Experiments

Experiment	Number of animals	Diuresis (in ml per 100 g weight per hour)	Urinary Na (in m-equivalents per liter)	Sodium excretion (in μ-equivalents per hour per 100g weight)
Control determination	32	0.049 ± 0.022	106	4.4
10% ascorbic acid, 1 ml per 200 g weight	32	0.200 ± 0.015	175	35.0
1% $NaHCO_3$ solution, 1 ml per 200 g weight	16	0.056 ± 0.030	131	7.3
Water load of 5% body weight	16	0.58 ± 0.034	9	5.2

The effect of ascorbic acid is therefore specific, and is most likely due to its antihyaluronidase effect.

At present we can put forward no explanation of the mechanism of the increased sodium excretion caused by the antihyaluronidases. It is certain however that it is not due to a simple increase in urinary excretion. As the results given in the table show, a diuresis two and a half times that due to the action of ascorbic acid, induced by a water load, does not cause any increased excretion of sodium. The concentration of sodium in the urine during excretion following increased water intake is reduced approximately in proportion to the increased urinary excretion, so that there is practically no change in the amount of salt excreted.

SUMMARY

Experiments on albino rats have shown that the antihyaluronidases (ascorbic acid and heparin) increase diuresis by reducing water reabsorption. The effect accords with the idea that enzymatic depolymerization of the hyaluronic complexes in the walls of the tubules is the cause of facultative reabsorption. Neutralized ascorbic acid markedly increases the excretion of sodium, but the mechanism is obscure.

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THE REFLEX PATHWAYS OF THE ANTICOAGULATION SYSTEM

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 52, No. 7, pp. 5-9, July, 1961
Original article submitted July 11, 1960

It has been shown [1-4] that the injection of moderate doses of thromboplastin or thrombin suppresses blood clotting, either partially or completely. A study of the blood shows that in response to the injection of thrombin into the blood stream substances are liberated which increase fibrinolytic activity and markedly reduce the amount of fibrinogen; there is also an increase of the concentration of heparin and heparinlike substances. This defensive reaction against the formation of thrombin is blocked by anesthesia, and if an injection of thrombin is given to an animal which has been deeply anesthetized for 15 minutes, it will usually die from thrombosis, whereas control animals will survive. The defensive reaction has been shown to exist not only in mammals, but in certain amphibia (in Rana temporaria) [5]. The injection of thrombin into the frog ventricle does not cause thrombosis, but actually completely prevents blood from clotting in a test tube when thromboplastin from the lungs from the same species of animal is added [5]. If, however, the spinal cord of the frog is destroyed before injecting the thrombin into the heart, the animals die from generalized thrombosis [5]. Destruction or removal of the brain in Rana temporaria did not prevent the occurrence of the protective action against thrombin injected into the blood stream. All the above results indicate that the protective reaction which maintains the blood in a fluid condition in vivo, is reflex in nature [1-5].

We here report further experiments which also indicate the reflex nature of the anticoagulation system.

METHOD

Experiments were performed on white rats weighing 170-180 g. They were kept on the normal laboratory diet. Blood was collected in a syringe from the jugular vein, and intravenous injections of thrombin were made into the same vein. The thrombin used was prepared by the N. F. Gamalen Institute of Epidemiology and Microbiology. The activity of the thrombin solution was determined from the clotting time of oxalated rat blood at 37°, when 0.3 ml of blood was added to 0.3 ml of thrombin.

RESULTS

As has been shown previously [5], in frogs, the reflex anticlotting system does not operate when the spinal cord has been destroyed. In rats we found that destruction of the cord at or below the VII vertebra causes a rapid reflex reaction which produces an anticlotting effect (Table 1).

It was found that the impairment of the blood-clotting power was of the same kind as was produced by intravenous injection of thrombin (Table 2). There was then a marked increase in the fibrinolytic activity of the blood, and a fall in the amount of fibrinogen. Blood taken after partial destruction of the spinal cord, and after intraperitoneal injection of thrombin, did not clot in vitro, and clotted only very slowly after thrombin had been added. However, the thrombin time of the same blood was greatly reduced if it was first incubated with protamine sulfate (Table 3); this result appears to indicate that there is an increased concentration of heparin or of heparin-like substances in the blood stream even after an intravenous injection of thrombin, and after the spinal cord has been destroyed.

It is possible that this reaction occurs through the formation in the plasma of antithrombin VI [6]. After partial destruction of the spinal cord the normal clotting power of the blood is restored after 60-90 minutes or more, and after this time intravenous injection of thrombin will again produce a complete protective reaction (Figs. 1 and 2).

TABLE 1. Determination of the Mean "Natural" Clotting Time of Rat Blood Before and After Destruction of the Spinal Cord*

Number of animals	Experimental conditions	Time of clotting for 0.5 ml of blood	Thrombin time (0.2 ml thrombin + 0.2 ml oxalated blood)
42	Before destruction of the spinal cord	80 sec	28 sec
42	After destruction of the spinal cord	From 3 min to more than 30 min	From 3-4 min to more than 10 min

* The blood was incubated in a glass tube with thrombin at 37°.

TABLE 2. Measurement of the "Natural " Clotting Time of Rat Blood

Number of animals	Experimental conditions	Clotting time for 0.5 ml of blood	Thrombin time (0.2 ml oxalated blood + 0.2 ml thrombin) *
31	Before intravenous injection of thrombin	On average, 79 sec	On average, 29 sec
31	Four minutes after the intravenous injection of thrombin (0.9 ml active, 5-6 sec)	From 4 min to more than 30 min	From 5 min to more than 30 min

* The blood was incubated in a glass tube with thrombin at 37°.

TABLE 3. Determination of the Thrombin Time of Acidified Rat Blood Incubated with Protamine Sulfate or Physiological Saline*(Mean Values)

Number of animals	Conditions of experiment	After incubating 0.2 ml of blood + 0.2 ml of thrombin with physiological saline	After incubating 0.2 ml of blood + 0.2 ml of thrombin with protamine sulfate
37	Before partial destruction of the spinal cord	28 sec	22 sec
37	4 min after partial destruction of the spinal cord	From 3 to more than 10 min	52 sec
28	Before intravenous injection of thrombin	29 sec	24 sec
28	After intravenous injection of thrombin	From 5 to more than 30 min	81 sec

* Other procedures were followed: 0.3 ml of blood + 0.03 ml of 0.2% protamine sulfate solution or 0.03 ml of physiological saline were incubated for 15 min at 37°.

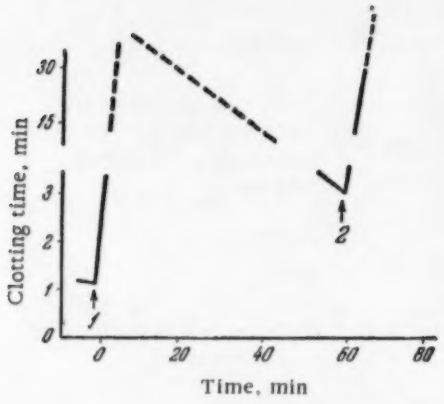

Fig. 1. Changes in the clotting time in rats caused by partial destruction of the spinal cord and subsequent intravenous injection of thrombin (mean results of 11 experiments). 1) Destruction of the spinal cord; 2) injection of thrombin.

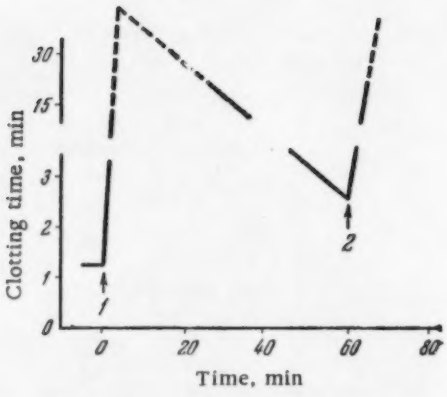

Fig. 2. Changes in the clotting time in rats induced by intravenous injection of thrombin and subsequent partial destruction of the spinal cord (mean values of 5 experiments). 1) Injection of thrombin; 2) destruction of spinal cord.

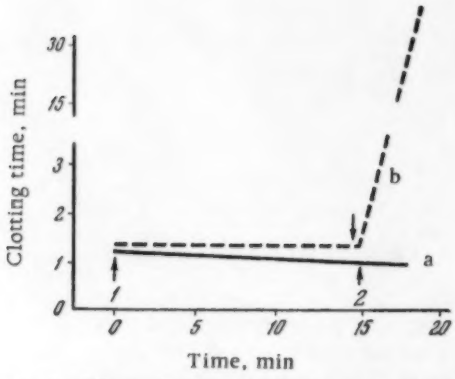

Fig. 3. Effect of ether anesthesia on changes in the clotting power of rat blood caused by destruction of part of the spinal cord (mean results of 24 experiments). a) Experiment; b) control (no anesthesia); 1) anesthesia; 2) destruction of the spinal cord.

The reflex-humoral reaction which leads to the loss of blood clotting power, and which is caused in rats by the partial destruction of the spinal cord, is completely blocked by anesthetizing the animals completely with ethyl sulfate at the time of the operation (Fig. 3).

Destruction of the thoracic and cervical portions of the cord (while respiration is maintained artificially) and destruction of the whole of the brain do not impair the activity of the anticlotting system. Experiments on rats in which the neocortex has been removed have shown that the anticlotting system is not destroyed, although it is somewhat weakened. Intravenous injection of thrombin caused a typical change in the anticlotting system, but unlike the operated controls which received an injection of inactivated thrombin, they frequently died within 20-30 min. Destruction or section in the region of the diencephalon or hypothalamus does not block the action of the anticlotting system.

However, section in the dorsal part of the cerebellum and medulla, while respiration is maintained artificially, immediately eliminates the anticlotting system; in such animals, controls which receive thrombin inactivated by heating survive, whereas those which receive an injection of thrombin die rapidly from a generalized thrombosis. In all cases, the operation completely blocks the anticlotting system. However, at the time at which the brain is sectioned, there is a weak reflex response, which influences the anticlotting system, and there is some increase in the clotting time and an increase in the fibrinolytic activity of the blood; this effect has some influence on thrombin injected subsequently, but it affords no protection from the thrombosis.

In later experiments it was shown that the reflex arc of the anticlotting system involves the medulla. A detailed account of this work will be published later.

SUMMARY

Further evidence has been obtained that the physiological anticoagulation system in rats is of a reflex-humoral nature. The reflex arc involves the medulla oblongata.

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THE EFFECT ON CARDIAC REFLEXES OF REMOVAL OF THE CHROMAFFIN TISSUE

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 52, No. 7,
pp. 9-14, July, 1961
Original article submitted July 5, 1960

Adrenalin is the precursor for the synthesis of the sympathetic nervous mediator sympathin. From experiments on various animals, removal of large amounts of chromaffin tissue reduces the amount of adrenalin in the blood, and so impairs sympathetic function in different organs [3]. This method of interfering with the synthesis of sympathin in order to assess its physiological role may also be used to determine its effect on the cardiosympathetic nerves, and to study the trophic effect of such nerves, which exert a stimulating effect on the heart [4-7,9]. In experiments in which large amounts of chromaffin tissue were removed from the adrenal glands [2,3] it was found that there were also certain changes in higher nervous activity: there was a reduced internal inhibition and an overt or concealed weakening of the process of stimulation; the same operative measure reduced the frequency of occurrence of the cardiac component of the conditioned defensive reaction [10].

The aim of the present work has been to study the part played by the chemical mediator sympathin in conditioned reflex effects affecting the heart, and in particular to find its effect on the rate and strength of the cardiac contractions.

METHOD

The experiments were carried out on five dogs over a period of 5-6 months. Cardiac contractions were recorded by means of a balloon; the thin-walled rubber balloon was introduced through a fistula into the pericardial cavity, and connection to a Marie's capsule was made by means of an air and water system. First a pericardial fistula was established using a modified version of N. P. Sinitsyn's method [8]. It was then possible to record not only the rate, but also the amplitude of the cardiac contractions. The amplitude can not be recorded by most of the methods applicable to chronic preparations, such as electrocardiography, or recording the blood pressure. The method is therefore important not only for the purpose of the present study, but also as enabling changes in frequency and amplitude of the heart beat to be recorded in chronic preparations. After the animals had become used to the experimental set-up, conditioned reflexes to a sound stimulus, consisting of a moderately large electric bell, were established. A special square-wave generator in which the frequency and voltages of the pulses could be controlled was used to supply the unconditioned reinforcement. The stimulating current was supplied to the shaved surface of the upper third of the left forelimb. At first, the experiments were performed on unoperated dogs, and subsequently were continued for long periods after the operation. In four animals, in order to cause a temporary impairment of the synthesis of sympathin, the right adrenal was removed, and the medulla of the left adrenal was cauterized. Two dogs served as controls: in one, to compensate for the removal of the adrenal medulla, injections of 1 ml of 1: 1,000 adrenalin were given daily, and in the other a mock operation was made in which the abdominal cavity was opened without the adrenal glands being touched, and the wound in the anterior wall was sewn up in layers.

RESULTS

In response to the signal alone, which accompanied the unconditioned pain stimulus, in all the dogs, usually after 10-14 combined applications (and in one after the third combined application) both the amplitude and frequency of the heart beat increased. The increase in amplitude was made greater by the application of the unconditioned stimulus (Fig. 1).

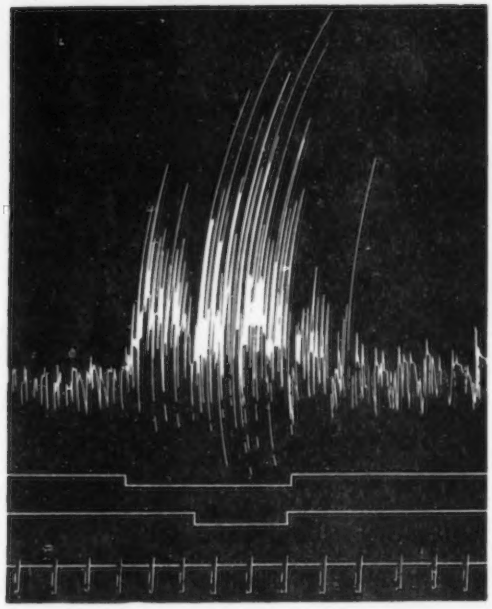

Fig. 1. Change in cardiac activity due to conditioned and unconditioned stimuli. Curves, from above downwards: cardiac contractions; conditioned stimulus marker; unconditioned stimulus marker; time marker (3 seconds).

After the conditioned reflexes had been well established, the greater part of the chromaffin tissue was removed, and three days after the operation systematic studies were resumed. In spite of individual differences, it was nevertheless possible to make out the general direction of the changes in the regulation of cardiac activity.

In all the operated dogs, including the controls, 3-5 days after the operation there was a disturbance of the conditioned reflex cardiac regulation. The cardiac response to the conditioned signal was either weak, or did not occur at all; in some cases it could be elicited only by two or three repetitions of the stimulus. Because there could be no profound impairment of the formation of sympathin soon after the operation [3], we attributed these changes not to the specific operation, but to the general postoperative effects due to anesthesia and trauma.

This explanation was confirmed by experiments on the control dog; after laparotomy performed without removing any adrenal medulla, the cardiac conditioned reflexes were absent for 2-3 days (from 3 to 5 days after the operation), but subsequently returned. Similar results were noted in experiments on the second control dog, in which the chromaffin tissue of the adrenals was removed, but which received compensatory injections of adrenalin. The similarity of the results obtained on the two dogs during the first few days after the operation suggested that the reduction or complete absence of cardiac conditioned reflexes was simply due to the trauma caused by the operation. The disturbance in the reflexes concerned only the response of the heart to the conditioned signal; the response to the unconditioned stimulus was normal: there was a regular increase in strength and frequency of the heart beat in response to a painful stimulus. Evidently the operative trauma does not noticeably affect the heart's response to unconditioned stimuli.

Later, after the removal of the adrenal medulla (from the 5-6th day onwards) there was a disturbance of the conditioned and unconditioned cardiac regulation. There was now a difference between the experimental and control groups in the responses to both conditioned and unconditioned stimuli; the rate and rhythm of the cardiac contractions were affected differently. The first effect was on the strength of the contractions. The changes were phasic: the first phase lasted 8-15 days, and the second for 10-11 days, while the third commenced 20-28 days after the operation. In the first phase, the reflex effect on the strength of the contractions was weaker than it had been before. During the next phase it was stronger: 20-28 days after the operation the strength of the response had returned to normal and was maintained at this level for the whole of the period for which observations were made.

The effect on the strength of the contractions in response to the unconditioned stimulus, which began on the 5-6th day, became reduced, and remained so for a long time. Recovery to the preoperational level in response to the unconditioned stimulus was not observed until the 52-62nd postoperational day.

In comparing the effects of the conditioned and unconditioned stimuli on the strength of the cardiac contractions, the extent and the duration of the reduction of the effect were most clearly shown in the case of the unconditioned stimulus. The graph shown in Fig. 2 illustrates this point; it shows the change in the strength of the contractions in dog No. 1, while Fig. 3 shows the results for dog No. 2.

No differences between the experimental and control groups were found in the change in the amplitude of the cardiac contractions. The only effect observed was that already described, and which occurred in the first few days after the operation. It should also be noted that the increased frequency of the contractions which occurred in animals from which the adrenal medulla had been removed showed some changes: the marked increase which occurred on certain days after the operation was followed by a period when no appreciable changes in rate occurred.

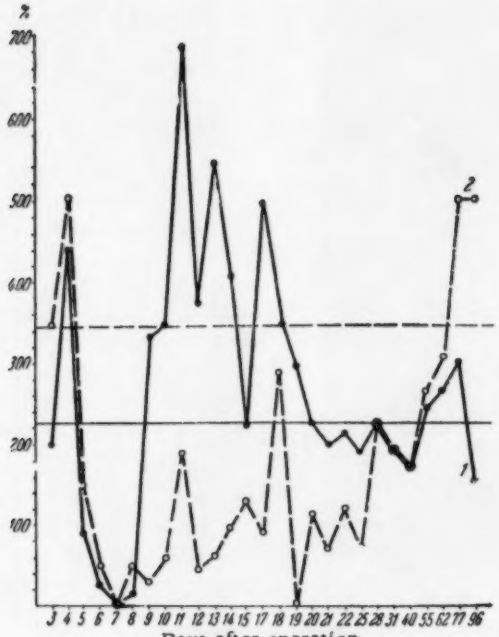

Fig. 2. Changes in the extent to which the strength of the cardiac contractions alters in response to conditioned and unconditioned stimuli, at different times after removal of the adrenal medulla. 1) Change in the amplitude of the cardiac contractions in response to the conditioned stimulus; 2) same in response to the unconditioned stimulus; abscissa– days after the operation; ordinate-change in the amplitude of the cardiac contraction expressed as a percentage of the amplitude before stimulation.

Thus, removal of the adrenal medulla causes a considerable effect on the nervous regulation of the amplitude of the heart beat, but has little effect on the regulation of the frequency. The continuation of the normal effect on heart rate in response to the conditioned stimulus could be found even when the adrenergic mechanisms were blocked by aminasin [1].

Elimination of most of the chromaffin tissue reduces the amount of adrenalin in the blood stream, and appears to prevent the synthesis of sympathin; the effect is shown in the reflex regulation of cardiac activity. The chief effect is on the control of the amplitude of the cardiac contractions. This effect was shown by the dog which received adrenalin after removal of the adrenal medulla. Here, there was no reduction in the control over the amplitude of the cardiac contractions for either the conditioned or the unconditioned stimulus. On the other hand, in response to a conditioned signal given after the 6th day, or to an unconditioned stimulus after the 19th day, there was a greater change in the amplitude of the contractions than had occurred before the operation.

SUMMARY

Conditioned reflex changes of the rate and intensity of the cardiac contractions were altered by the removal of the adrenal medulla. Studies were made on chronic preparations of dogs having a pericardial fistula. The conditioned cardiac reflexes were reduced or disappeared completely at the third, fourth, and fifth postoperative days. The amount by which the amplitude of the contractions changed in response to the stimulus was different on the

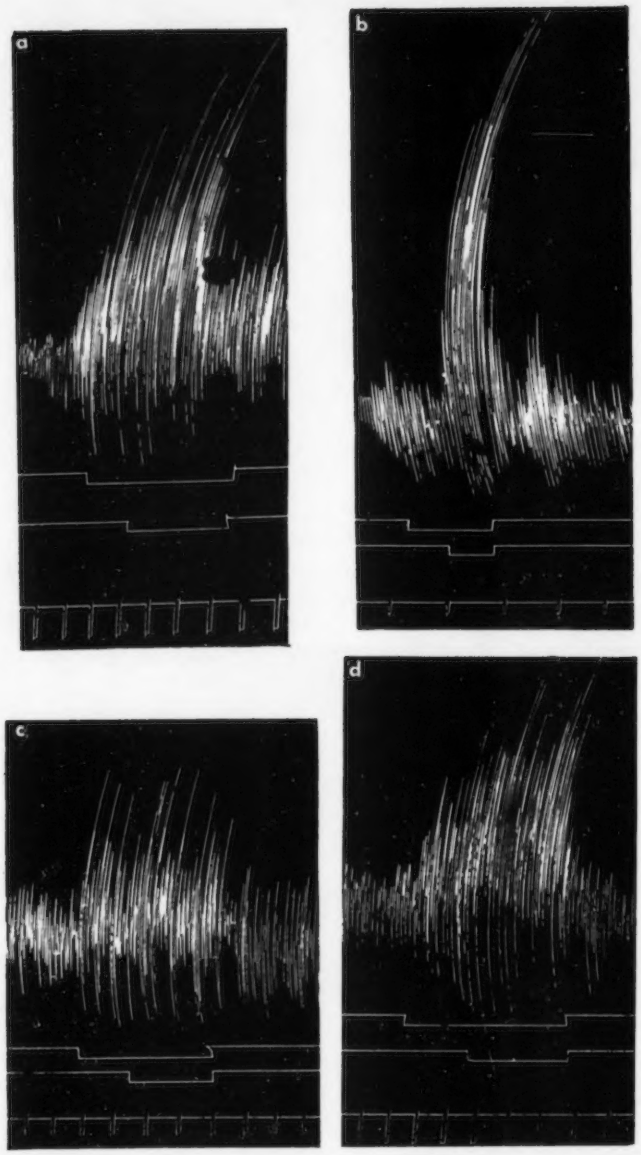

Fig. 3. Change in cardiac activity in response to conditioned and unconditioned stimuli at different times after removal of the adrenal medulla. a) Before operation; b) on the 4th day after operation; c) on the 11th day; d) on the 26th day. Curves, from above downwards: cardiac contractions; conditioned stimulus marker; unconditioned stimulus marker; time marker (3 seconds).

fifth and sixth postoperative days. The changes were phasic: first there was a reduction, then an intensification, and finally a gradual re-establishment of the preoperative level. In response to an unconditioned stimulus, the failure of the normal increase in cardiac contractions was more marked and prolonged. The removal of the adrenal medulla had no significant effect upon the elicitation of an increased heart rate.

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THE INFLUENCE OF UREA ON NERVOUS STIMULATION OF CARDIAC MUSCLE

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 52, No. 7,
pp. 15-17, July, 1961
Original article submitted July 5, 1960

Ya. P. Sklyarov and his co-workers have shown that metabolic products may affect the recovery processes in glandular tissue and in the heart [5-11].

We here report a study of the effect of the products of nitrogen metabolism (urea) on recovery in cardiac muscle. We chose urea, because according to Kh. S. Koshtoyants and his co-workers [4], this substance brings about the splitting off of free sulfhydryl groups from the protein molecule, and these groups play a part in the oxidation-reduction processes in tissues, and are also concerned in neurohumoral regulation.

METHOD

Two kinds of experiments were made: 1) 58 in which frog hearts were prepared by Saima's method, and the sympathetic and vagal supply retained for stimulation, and 2) 13 acute experiments on dogs in which records were made of the blood pressure and pulse pressure. We first found the effect of a 0.5-1% urea solution on the isolated frog heart, and of a 20-40% urea solution on the blood pressure of the dog. Then, in all experiments, a control stimulation was made of the vago-sympathetic or sympathetic nerves, by means of an induction coil. A study was then made of the effect of nervous stimulation of the dog heart; this was done during perfusion of the isolated heart with urea, or after injecting urea intravenously.

RESULTS

Perfusion of the isolated frog heart with 1-2 ml of 0.5 and 1% urea solutions led to an increase in the amplitude of the contractions, and in some experiments also increased the rate. The effect did not last for more than 1-2 minutes. Intravenous injection of 0.5-1 ml per kg of 20-40% urea solution caused a transient reduction in blood pressure and some increase in the pulse pressure.

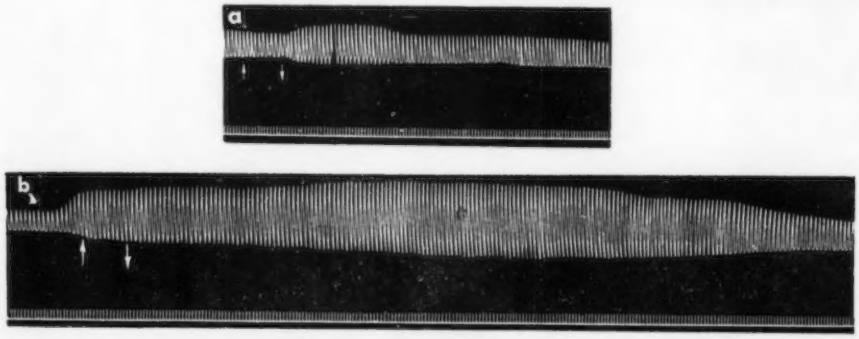

Fig. 1. Stimulation of the sympathetic nerve supply to the frog heart. Experiment made on 23/12/1956. (Distance between the coils of the induction coil – 12 cm; voltage applied to primary winding- 6v.) a) Control; b) after perfusion with 2 ml of 1% urea. Curves, from above downwards: cardiac contractions; time marker (1 second); arrows – time at which nerve was stimulated.

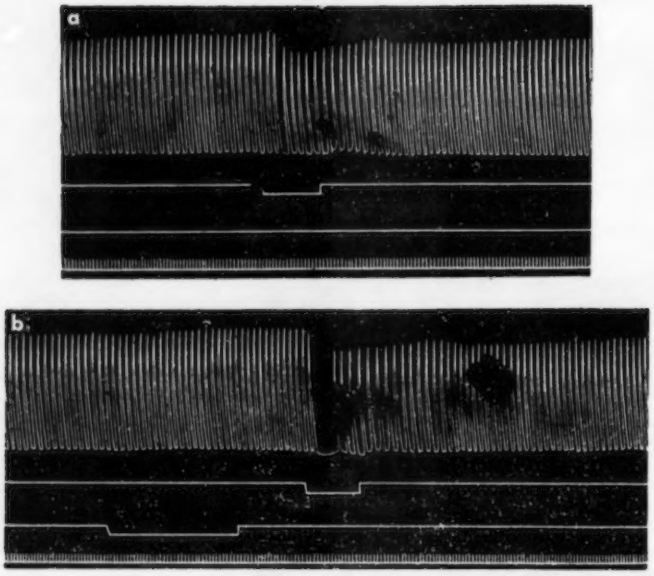

Fig. 2. Stimulation of the vagosympathetic nerve in the frog. Experiment made on 20/1/1956 (distance between coils 8 cm, voltage applied to the primary 6 v). a) Control; b) after perfusing the heart with 2 ml of 1% urea solution. Curves, from above downwards: cardiac contractions; stimulation of the nerve; perfusion with the solution; time marker (1 second).

Perfusion of the isolated frog heart with 0.5 and 1% urea solutions markedly increased and prolonged the positive inotropic effect (Fig. 1, a, b).

Similar changes were observed in the experiments on dogs. After intravenous injection of 10 ml of a 40% urea solution, the hypertensive effect of stimulating the sympathetic nerve was more clearly shown. These results agree with those of Kh. S. Koshtoyants [3] on the fatigued striated frog muscle. He found that a given strength and duration of stimulus applied to the sympathetic nerve supplying a fatigued muscle treated with urea caused a far greater increase in the height of the contractions than it did when the muscle was immersed in Ringer solution.

In the presence of urea, the effect of vagosympathetic stimulation is different from that in the control animal (Fig. 2, a, b). When the heart is perfused with urea, the inhibitory effect from the vagus is enhanced.

A similar effect was observed also in acute experiments on dogs. After injecting 20% urea solution intravenously, the depressor vagosympathetic action was enhanced, and it lasted longer than it did in the control experiment. This result agrees with those of Kh. S. Koshtoyants, T. M. Turpaev and K. S. Logunova [1,2], who found that urea restored the inhibitory effect of the vagus, which is destroyed when sulfhydryl groups become bound by thiol poisons; they found that it also eliminated the phenomenon of "escape" of the heart from vagal inhibition.

Urea therefore enhances the effect of sympathetic and vagal stimulation in both the frog and dog. A possible explanation is that the functional change is due to the liberation of active sulfhydryl groups from the protein molecules, and that these groups influence the cardiac response to neurohumoral action.

SUMMARY

A study was made of the effect of urea on regeneration in cardiac muscle. Urea was chosen because according to results obtained by Kh. S. Koshtoyants, this substance promotes the liberation of free sulfhydryl groups from the protein molecule, and so plays an important part in the oxidation-reduction processes in the tissues, and in neurohumoral regulation.

The experiments reported here were carried out on isolated frog hearts, and the sympathetic and vagus nerves were retained for stimulation. Blood pressure and fluctuations in pulse amplitude were recorded in acute experiments on dogs. Usually, the vagosympathetic or sympathetic nerves were first stimulated by current from an induction coil. A study was then made of the specific features of the effect exerted by these nerves on an isolated frog heart perfused by 0.5-1% urea solution, or on the heart of an intact animal which was given an intravenous injection of 20-40% urea solution. In evaluating these results, attention was paid to the frequency and amplitude of the cardiac contractions, and in dogs to the blood pressure and pulse pressure. Perfusion of the isolated frog heart with 1-2 ml of 0.5 or 1% urea solution caused transient changes in both the amplitude and the rate of the heart beat, but intravenous injection of 0.5-1 ml per kg of 20-40% urea solution caused no significant drop in blood pressure but produced a rise in the amplitude of the pulse pressure. Treatment with urea considerably intensified the effect of stimulation of the cardiac sympathetic and vagosympathetic nerves.

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ADAPTATION OF THE VISUAL APPARATUS TO NATURAL ILLUMINATION

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 52, No. 7,
pp. 18-20, July, 1961
Original article submitted July 22, 1960

The lability of the visual analyzer [1-6] has been studied in terms of changes in the areas of light and color sensitivity, the number of microscotomata, the size of the blind spot, the threshold size of the area stimulated, and dark adaptation curves taken at different times of day. By this means it has been found how the retina becomes reorganized to adapt itself to the illumination by altering the mobilization of the photoreceptors.

The aim of the present study has been to examine the adjustment of the visual apparatus, and its reorganization during the changes of illumination occurring at sunset.

METHOD

Light thresholds were measured binocularly by means of an A-M adaptometer. No preliminary adaptation was given, in order that the measurements should reflect the correspondence between the organization of the eye and the current illumination. We made 40 observations on five subjects. In each experiment, the light sensitivity was measured every 3-4 minutes for 1 hour before sunset, and for 15-20 minutes after. The measurements were repeated several times during the day. Measurements of the light intensity were made by the luxmeter. The work was carried out in November and in February, when sunset occurred between 4.06 and 6.02 p.m.

RESULTS

A rapid increase in light sensitivity began 5-8 minutes before the sun had completely set. The maximum increase was at the actual time of sunset, though it might occur 3-5 minutes afterwards, or 3 minutes before; the peak increase in sensitivity therefore did not always coincide with the moment of sunset. When the sun had completely set, color sensitivity remained at a higher level.

We will give as an example the results obtained on subject A (Fig. 1).

Measurements of light sensitivity were made at intervals during the day, and at 40 minutes before sunset, which occurred at 4.06 p. m.

The mean values of the light sensitivity for the corresponding arbitrary time intervals were taken at 11 a.m., 12 noon and 3 p.m. The values from 3.30 p.m. to 4.30 p.m. showed the changes in light sensitivity which occurred during the experiment before and after sunset.

Fig. 1 shows that the relatively stable light sensitivity level underwent a sudden sharp increase, which in this case occurred 7 minutes before sunset, and which attained a maximum value at the time of sunset itself.

After the sun had set, the sensitivity was several tens of times higher than before.

In the subject Ch light sensitivity measurements were started 63 minutes before sunset (5.43 p.m.) and were continued for 17 minutes after. Fig. 2 shows that the relatively stable level of light sensitivity before sunset was followed by a marked increase, which occurred 8 minutes before sunset. The maximum increase in the light sensitivity was attained at the moment of sunset itself, and was maintained subsequently.

Therefore, before sunset the retina undergoes a gradual reorganization to twilight vision, and after the whole of the sun's disk has sunk below the horizon, a sudden increase in sensitivity is added on to the effect of the continuous adaptive process. The increase in light sensitivity during the decrease in illumination from the sun during sunset

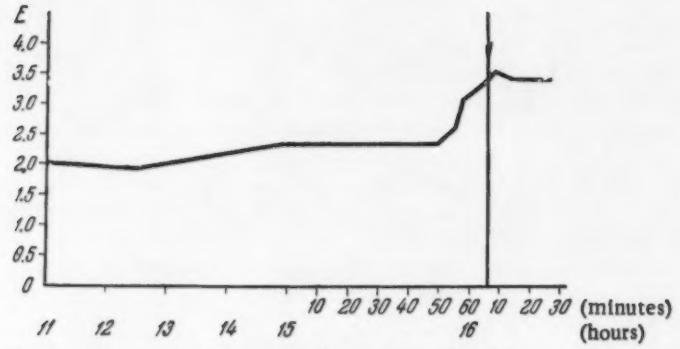

Fig. 1. Changes in light sensitivity at sunset in subject A. Abscissa-time of observation (from 11 a.m. to 3 p.m., and from 3 p.m. to 4.30 p.m.); ordinate - light sensitivity in logarithmic units (E). The arrow indicates the time of sunset (4.06 p.m.).

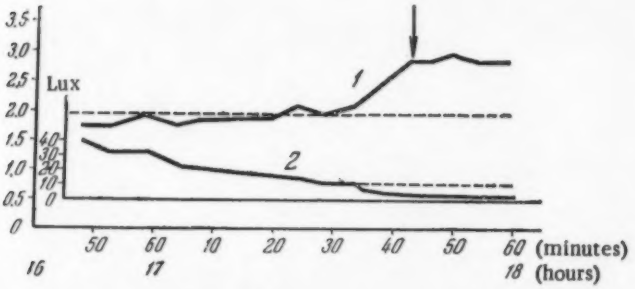

Fig. 2. Changes in the light sensitivity (1) in logarithmic units (E) and figures for intensity of illumination (2) in lux during the period in which light sensitivity changes occur. The arrow indicates the time of sunset (5.43 p. m.)

causes an increased activity of the retinal rods, and during the evening they become mobilized maximally. These observations also show that increase in retinal sensitivity can occur without previous dark adaptation, and therefore that under normal conditions of illumination the retinal receptors are in a condition in which they are ready to be mobilized.

Therefore, at sunset, the retinal rods become mobilized and their sensitivity increases rapidly to a maximum value before sunset. The peak light sensitivity occurs either at the moment of sunset, or 3-5 minutes previously, and remains at this level subsequently. This visual adaptive reaction has been brought about during the course of evolution and has been reinforced during the lifetime of the individual, and represents the biological means whereby the receptors become adjusted to external illumination. This index may be used as a clinical functional test for a broader study of the condition of the visual system.

SUMMARY

It was shown that natural illumination determines retinal adaptation. The moment of special interest occurred at the change from daylight to twilight vision while the person under observation was in natural conditions. Measurement of the retinal light sensitivity showed that the change from daylight to twilight vision was brought about by an increased sensitivity and activity of the rods which occurred after sunset.

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MEASUREMENTS OF THE ELASTICITY OF THE MAMMARY GLAND AS RELATED TO THE HUMAN MILK EJECTION REFLEX

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(Presented by Academician V. N. Chernigovskii)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 52, No. 7, pp. 20-23, July, 1961
Original article submitted June 17, 1960

A. G. Ginetsinskii, V. F. Vasil'eva, M. G. Zaks, M. M. Sokolova, and V. A. Soo [1] have described how an elasticity measurement may be used to study the capacity of the human breast; the method was applied by V. A. Soo [3] to study the relation between pressure in the gland and the milk content, and pressure changes caused by pituitrin, which caused a contraction of the alveoli. In the present work we have used this method to study the milk ejection reflex during breast feeding.

METHOD

Observations were made between the 5th and 19th day after the onset of lactation on 59 women who were feeding their babies. In 41 cases it was the first child which was being fed, and in 18 cases other children had been born previously. We used the device developed by A. G. Ginetsinskii and his co-workers [1] and previously employed by V. A. Soo [3].

The principle of the method was as follows. A small metal disk with a conical elevation in the center is applied to the skin of the gland. A pendulum which takes the form of a small hammer supported on a horizontal axis falls from a certain distance on to the cone of the disk, and rebounds after it has struck it. The duration of the contact is measured by means of the deviation of the mirror of a ballistic galvanometer connected in an electrical circuit which is completed by the hammer as it falls on the cone, and broken when it rebounds. The greater the pressure in the mammary gland, the shorter is the period of contact. Measurements were begun 15-20 minutes before feeding.

The disk was applied to the breast from which the infant was to feed; then six to eight changes in pressure were rapidly applied at approximately 1 minute intervals. The disk was then transferred to the opposite gland, from which the infant had fed the previous time, and a further six to eight corresponding measurements were made. The infant was then handed to the mother, and after the breast had been prepared in the usual way, breast feeding commenced. During the whole of the feeding time, and 8-10 times afterwards, pressure measurements were made in the opposite breast. The disk was then again transferred to the opposite side, and the measurements repeated on the breast from which the infant had fed. The amount of milk taken was measured by weighing the infant.

RESULTS

During feeding, considerable pressure changes are produced in the opposite breast.

Figs. 1 and 2 illustrate the most typical reactions.

In some cases (see Fig. 1) the pressure in the opposite breast increased rapidly, and then fell equally rapidly and remained low for the whole of the feeding time, and after feeding it rapidly returned to its original value. In other cases, there was again a rapid increase in pressure, which then underwent rhythmical changes, rising and falling alternately. At the end of feeding the pressure in the opposite breast returned, as before, almost to its

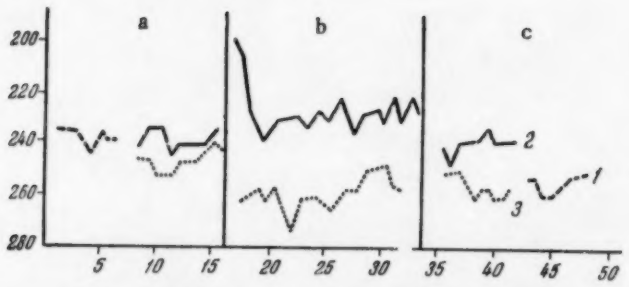

Fig. 1. Pressure changes in the mammary glands in subject I. A. during feeding, 6th and 16th days of lactation. Abscissa – time (in minutes); ordinate – pressure in the mammary gland expressed in arbitrary units (millimeters of the galvanometer scale). a,b,c) different periods of breast feeding; 1) change in the pressure in the breast in use before and after feeding; 2) same, on the opposite side on the 6th day of lactation; 3) response of the opposite breast on the 16th day of lactation.

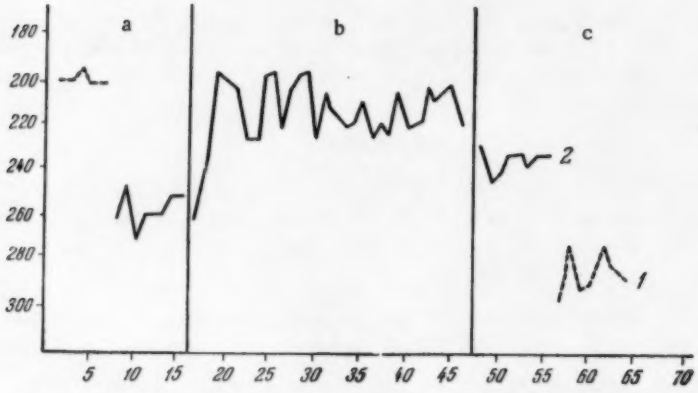

Fig. 2. Reaction of the mammary gland to feeding. Subject L. V., 7th day of lactation. Indications as in Fig. 1. 1) Change in tension in the breast in use, before and after feeding; 2) same,on the opposite side.

original value. Finally, in some cases (see Fig. 2) there was an initial quick rise, after which the pressure remained elevated, and continued elevated even after feeding had ceased.

According to reports, feeding causes an increased pressure in the opposite breast. In the publications to which we have referred, it was shown that this increase occurred either because of an expansion of the internal spaces of the gland (its volumetric system) caused by engorgement with milk, or, when the amount of milk remained unchanged, through a contraction of the contractile elements of the walls of the alveoli and ducts. Relaxation of the contractile elements or emptying of the gland causes a reduction in pressure.

In our experiments, there was no reason to suppose that there was any appreciable change in the amount of milk on the opposite side. Therefore all the pressure changes which were recorded (either increases or decreases) were due to the tone of the contractile elements. The initial increase in pressure was evidently due to the oxytocin reflexly secreted by the neurohypophysis, which is stimulated by the act of breast feeding; the oxytocin reaches the alveoli, and stimulates the contraction of the myoepithelium. The subsequent response of the unused breast shows many individual variations, but there is a common tendency: the pressure returns to its original level, and the initial effect of oxytocin is not maintained. In some cases the readjustment occurs rapidly (see Fig. 1), in others it is less complete, and sometimes (see Fig. 2) there is hardly any stabilization. Presumably, this stabilization of the pressure in the unused breast is associated with the reflex reduction in the tone of its contractile elements in

response to the increased pressure in the volumetric system. A reflex relaxation effect of this kind has been studied by detailed observations on cows [2]. It has an important adaptive significance, because it prevents the development of an excessive pressure in the gland, which could prevent milk excretion.

The results we have obtained indicate that in women this relaxation reflex is very well developed. It may be that the ability to cause a great reduction in the tone of the contractile elements in response to an increased pressure compensates for the development of a moderate pressure in the volumetric system.

The three characteristic types of reaction of the unused breast indicate apparently that the unconditioned relaxation reflex may be developed to various extents during the first few days of lactation. The evidence is that as lactation develops, the reaction of the unused breast may change considerably. A typical example of such changes is shown in Fig. 1. It can be very clearly seen that during the ten days which elapsed between the first and second measurements, the relaxation reflex has become considerably enhanced; consequently feeding takes place without any appreciable increase in pressure occurring in the opposite breast.

We must also consider the pressure changes occurring in the used breast after feeding. V. A. Soo [3] has shown that the pressure increases in the time intervals between feeding, and falls considerably immediately after the breast has been emptied. However, she did not investigate the direct relationship between the amount of milk removed from the gland during feeding and the gradual fall in pressure. We have made such measurements, and they are shown in Fig. 3.

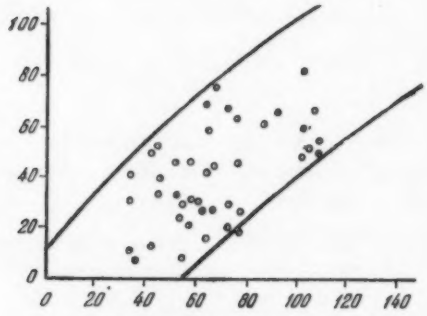

Fig. 3. Relationship between the fall in pressure and the amount of milk withdrawn. Abscissa – amount of milk withdrawn by infant (in ml); ordinate – fall in pressure in the breast after feeding (in mm of the galvanometer scale).

The relationship between the amount of milk removed by the infant and the fall in pressure is very clearly shown. Naturally, there is a considerable variation between different experiments, but the limits of these variations are very clearly defined, and the nature of the relationship is well shown.

Our results therefore confirm the work of V. A. Soo, who proposed that the rise in pressure in the mammary gland after feeding is due to the accumulation of milk.

SUMMARY

Mammary gland function was studied by measuring its tension. The changes in tension were measured during milk discharge. At first the tension in the opposite gland was found to rise, but it then fell to approximately the initial level. The pressure reduction is due to a special relaxation reflex which prevents the development of an excessive pressure. A relationship was established between the elastic tension in the gland and the amount of milk removed from it.

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INSULIN SECRETION BY THE PANCREAS OF THE CHICK EMBRYO

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 52, No. 7,
pp. 24-27, July, 1961
Original article submitted March 9, 1960

A number of morphological studies have been made of the development of the islets of Langerhans in the pancreas of chick embryos [4,7,8,10,13], which however give no clear idea of its secretory function. Also, as with other endocrine glands, to determine the part played by the islets in regulating the metabolism of the embryo, accurate information is required as to when the onset of their hormonal activity occurs. The most convincing evidence of secretory function of any gland is the identification of its hormone in the blood. Practically no observations of this kind have been made on the endocrine glands of embryos. In particular, no information is available on the time at which insulin occurs in the blood of chick embryos.

METHOD

For the detection of insulin we used a modern method—that of the isolated rat diaphragm [11]. Although the reliability for a quantitative estimation is somewhat doubtful, it was completely satisfactory for our purpose.

Fig. 1. Vessels for testing the absorption of glucose by the isolated rat diaphragm.

We used the method of Vallance-Owen and Hurlock [12]. Rats, which were mostly males weighing 120-150 g, were starved for 16-18 hours, and then decapitated. The diaphragm was then excised with a small scalpel, and immediately placed in saline cooled on ice [5], containing no glucose, and prepared with twice-distilled water. The diaphragm was lightly rinsed in the solution, and then laid out on filter paper placed on a glass plate and moistened with the same cooled solution. The posterior part of the diaphragm and its fibrous portion were removed, and the remainder was cut as nearly as possible into two equal portions. Both halves were immersed in cooled saline and left on ice for 20-25 minutes. They were then removed, lightly dried with filter paper, and placed in two special vessels (Fig. 1), one of which contained saline mixed with 300 mg% glucose (2 ml), and the other the same solution to which insulin or plasma had been added (see below).

In the control experiments, both vessels were filled with saline and glucose. The vessels were plugged with a ground stopper; rubber tubes were connected to their outlets, and one served to connect the vessels to each other. They were then fixed to a stand made from the wooden portion of a Warburg manometer. The stand was fixed to a support, and a gaseous mixture of 95% oxygen with 5% carbon dioxide was passed through both vessels for 5 minutes (Fig. 2). The vessels were then closed by plugging the rubber tubes with glass stoppers, and were placed in the water bath of Warburg's apparatus at a temperature of 37.3-37.4°, where they were shaken at a rate of 110-130 strokes per minute. After 90 minutes, the vessels were removed from the water bath, dried with filter paper, and their contents estimated for sugar by the Hagedorn-Jensen method. Measurements were also made of the sugar content of the original solutions. The determinations were made in three parallel tests. After each experiment, the vessels were boiled in strong alkali, and treated with a chromic acid mixture.

Blood was taken from the embryos by a method described previously [3]. Heparin was added, and in order to maintain conditions constant, it was added in each case, irrespective of the age of the embryo and of the ability

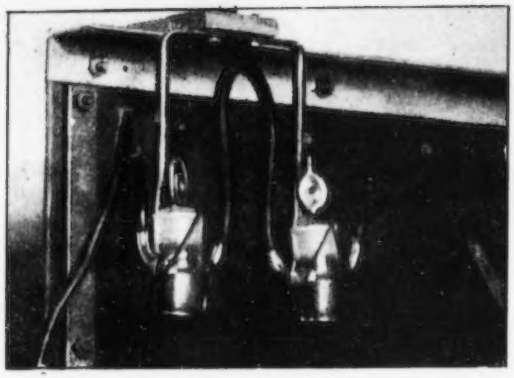

Fig. 2. Vessels fixed to a holder while the gaseous mixture is passed through. The gaseous mixture from the container passes successively through both vessels. The outlet tube is taken to a vessel containing water, which serves to verify that the gas is passing.

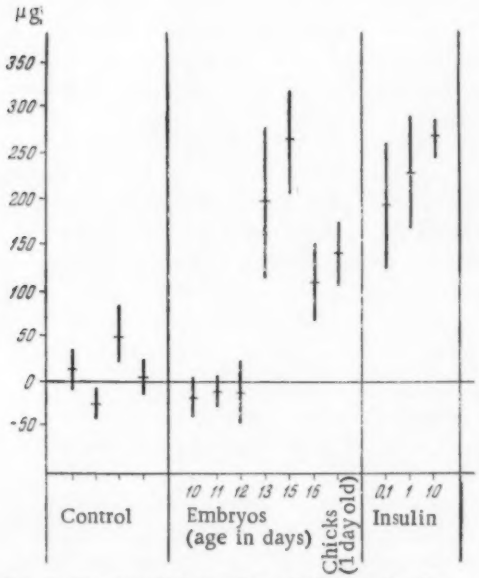

Fig. 3. Increased absorption of glucose by the diaphragm caused by insulin. Each vertical line represents the result of one experiment on 5-6 rats. The cross strokes in between the vertical lines represent the difference, in μg per 10 mg dry weight of diaphragm, occurring in 90 min between the amount of glucose absorbed by the half-diaphragm placed in the test solution and the other half placed in a pure saline solution of glucose. In the control experiments, both halves of the diaphragm were placed in the pure saline-glucose solution. The age of the embryos in days is indicated. One-day-old chicks were used. The insulin concentration is expressed in microunits per ml of the solution.

of the blood to clot. Blood samples collected from embryos of a given day of incubation were mixed; the plasma was separated by centrifugation, and diluted 10 times in saline containing 300 mg% of glucose.

In the experiments with insulin, we used a dry purified preparation, prepared from the insulin produced by the meat plant, and which contained 21 units per mg. It was diluted in a 0.6% solution of acetic acid, so that each ml contained 20 units. It was kept in this state in a refrigerator for two to three weeks. Before the experiment, this solution was diluted with saline containing 300 mg% of glucose; it was first diluted 20 times, and then, according to the concentration required, the dilution was made 10^5-10^8 times. Thus, the ultimate solution contained from 0.01 to 10 microunits per ml.

All the results were expressed as μg of glucose absorbed in 90 minutes per 10 mg of dry weight of diaphragm. Drying was continued at 105° until the weight remained constant, for which two hours was usually required. To determine the effect of plasma or insulin, the absorption in the solution under test was compared with the control solution, i. e., a comparison was made of the absorption of glucose by the two halves of the diaphragm. From the results obtained the mean difference and the standard error were calculated.

RESULTS

Of the large number of experiments which we performed we used only those of the last series, in which simultaneous measurements were made on six rats, or exceptionally on five.

The results are shown in Fig. 3.

They indicate that there is no insulin activity in the plasma of embryos less than 13 days old, and that it can be detected in embryos more than 13 days old. Evidently the pancreas begins to secrete hormone into the blood on the 13th day.

It is difficult to make a quantitative measurement of the insulin activity. Despite precautions, in the control experiments both halves of the diaphragm did not absorb the same amount of glucose. Although, in most cases, the divergence was insignificant, it was sometimes quite large. This effect would explain the quite large value of the standard error of the mean difference of the two absorptions.

In our experiments, we were unable to ensure that a single insulin or plasma solution should have the same effect in different rats. Consequently, in such experiments, the standard deviation of the mean was very high. The reason may have been the large variation in sensitivity of the rat to insulin. Unlike most other authors, we carried out the experiments on genetically inhomogeneous material. Possibly also, we may have failed to take account of some

undetermined experimental conditions which prevented us from obtaining uniform results. Therefore, at the present time, we have only an approximate knowledge of the activity of the plasma of chick embryos. When the plasma is diluted 10 times, its activity is approximately equal to 0.1-10 μ units/ml.

It should be noted that the activity of the plasma taken from 13-and 15-day-old embryos was greater than that obtained on later days. However, there is no reason to suppose that the effect is genuine, and not due to chance. A more accurate quantitative estimation of the insulin activity of chick embryos at different days of incubation must be made, and a great number of observations will be required.

We must also draw attention to the very great sensitivity of the rat diaphragm to insulin, as shown in our experiments. The average value of the excess absorption of glucose was much greater than that which has been reported by other workers. Thus, Wright [15], using an insulin concentration of 10 μ units/ml, found an excess absorption of glucose of 34 μg /10 mg /90 minutes; Vallance-Owen and Hurlock [12] obtained a value of 100 μg, and in our experiments the corresponding figure was 270 μ g /10 mg /90 minutes. The authors quoted give no figures for the effect of lower concentrations of insulin, and apparently there was no effect. However, we found a marked effect even at insulin concentrations as low as 0.1μ units/ml, and the effect did not disappear until the solution was diluted a further 10 times. It is not possible to explain the high sensitivity of the diaphragm, but the condition is favorable to the identification of insulin in blood.

Thus, our results indicate that in chick embryos insulin is secreted into the blood by the pancreas from the 13th day onwards.

This result is in line with the morphological descriptions of the islets of Langerhans [4,8], and agrees with the data on carbohydrate metabolism of the chick embryo. It is at the 13th day that appreciable amounts of glycogen begin to accumulate in the liver [1,6]. Long ago, Potvin and Aron [10] and later Needham [9] attempted to associate the formation of glycogen in the liver with the onset of pancreatic secretion, although later work showed that glycogen appears long before the islets of Langerhans begin to function. Possibly the onset of function is not associated with the appearance of glycogen in the liver, but with its rapid accumulation. This idea has been proposed by L. G. Leibson [1,2], Willier [14], and M. S. Mitskevich [4]. However, until now there were no facts to support it. The statement by Needham [9], based on a personal communication from Pecher and Hannon, that insulin may be detected in the embryo from the 11th day onwards, is contradicted by the fact that on the 11th day there is a reduction and not an increase of liver glycogen. Our results completely explain the sharp change in carbohydrate metabolism observed in chick embryos on the 13th day; we have been able to show that it is precisely at this stage that the pancreas begins to secrete insulin into the blood.

SUMMARY

Insulin activity of chick embryo plasma was determined by tests on an isolated rat diaphragm. The blood from several embryos was mixed; the plasma was separated by centrifugation, and was diluted 10 times with salt solution containing 300 mg% of glucose. The glucose absorption was expressed as micrograms per 10 mg (dry weight) of the diaphragm per 90 minutes. The insulin effect was measured by the difference between the amount of glucose absorbed by one of the halves of the diaphragm, immersed in a test solution, and the amount absorbed by the other half immersed in pure glucose solution. It was found that insulin did not appear in the plasma of the chick embryo until the 13th day. The insulin activity of plasma diluted 10 times was approximately 0.1-10 μ units per ml.

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THE EFFECT OF SHAM FEEDING ON THE FEEDING BEHAVIOR OF ESOPHAGOTOMIZED DOGS

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 52, No. 7, pp. 28-32, July, 1961
Original article submitted June 7, 1960

There is a close relationship between the drinking and feeding behavior of animals. I. V. Malkiman [8] pointed out that dogs kept short of food drank very little water. On the other hand, A. I. Lakomkin [7] showed that when drinking time is increasingly restricted, the amount of food consumed falls until finally none is accepted. I. N. Zhuravlev [6] found that when the dogs are sham-fed, water is drunk when the amount in the ration does not exceed the daily requirement. However, the daily requirement itself depends on many conditions, including the quality and quantity of the food consumed.

The receptors of the upper part of the digestive tract are well known to play an important part in the development of both positive and negative feeding and drinking responses. I. N. Zhuravlev showed that in dogs, the actual act of sham drinking of up to 10 liters of fluid may abolish the stimulus to drink. Even a very strong urge to drink may be eliminated by introducing large amounts of water in a balloon placed in the stomach, or by repeatedly pouring water directly into the stomach. A. I. Baranov [1] and I. P. Bel'skaya [2] inflated a rubber balloon introduced into the stomach of an esophagotomized dog through a gastric fistula. They found that the positive feeding response was obliterated, but that it was renewed when the balloon was withdrawn.

There is therefore a resemblance between the feeding and drinking responses under sham conditions.

We set out to observe the simultaneous drinking and feeding responses, under conditions of fractional true and fractional sham feeding, in order to determine the part played by the receptors on the upper portion of the digestive tract during the interaction of the feeding and drinking reactions.

METHOD

We carried out 40 experiments on fractional true and fractional sham feeding, under conditions where drink was given freely. The food stimulus consisted of crusts of white bread given in 10 g portions, or of boiled liver or spleen given in 30 g portions. The crusts and meat were finely chopped. A portion of the food was offered at intervals of five minutes. Water was placed on the stand, and the dog could drink it at will. Three experiments in which fractional feeding was given were performed while water was withheld. The experiments in fractional true feeding were carried out on three dogs, and those on fractional sham feeding on a single dog (Mal'chik) after esophagotomy; in this dog, a gastric fistula was established. All the experiments were carried out after a 16-24 hour period of fasting. Before the experiments, a diet for each dog was worked out which ensured that the weight (9-10 kg) remained unchanged. There was no restriction on fluid intake.

During the experiment, a determination was made of the time for which the animal continued to eat "until refusal", i. e., of the time for which the positive feeding reaction was maintained, and the amount of food and water consumed was noted. A record was also made of the behavior of the animal during the experiment.

RESULTS

With fractional true feeding with crusts, the amount of water drunk was greater, the greater the amount eaten. In three experiments, the dog Zhuchka ate 370 g of crusts and drank 750-850 ml of water. The greatest amount of water drunk was 1,000 ml, when it consumed the greatest amount of crusts (410 g) (Table 1).

Two experiments were carried out on the dog Mal'chik in such a way that the same amount of crusts (300 and 310 g) was consumed in different times, because in experiment No. 1, the portions weighed 5 g, whereas in experiment No. 3 they weighed 10 g. It was found that more water was drunk in experiment No. 1, which lasted consider-

TABLE 1. Drinking Reaction in Dogs during Fractional True and Sham Feeding (Crusts and Bread)

Number of Experiment	Name of dog	Amount of food consumed (in g)	Time of eating until refusal of food (in minutes)	Feeding reaction	Drinking reaction
	Fractional true feeding with crusts, while drink is given freely				
11	Zhuchka	370	185	Food refused	Drank 850 ml
13	"	370	185	" "	" 750 "
16	"	410	200	" "	" 1000 "
17	"	390	195	" "	" 900 "
20	"	370	185	" "	" 800 "
1*	Mal'chik	300	300	No refusal	" 1000 "
3	"	310	170	" "	" 750 "
2	Belyi	150	105	Food refused	" 250 "
	Fractional true feeding with crusts without fluid				
4	Zhuchka	110	50	Food refused	
14	"	270	150	" "	
	Fractional sham feeding with crusts				
6	Mal'chik	270	165	Food refused	Drinks a little
7	"	410	210	No refusal	Does not drink
8	"	80	40	Food refused	" "
9	"	100	50	" "	" "
10	"	100	50	" "	" "
11	"	60	30	" "	" "
12	"	70	35	" "	" "
13	"	80	35	" "	" "
20	"	Not recorded	45	" "	" "
	Fractional sham feeding with bread				
18	Mal'chik	490	85	Food refused	50 ml drunk

* Portion of crusts weighed 5 g, not 10 g.

ably longer (see Table 1). The same effect occurred in all the experiments with Zhuchka.

In the experiments with fractional true feeding with meat, we found no definite relationship between the amount of food consumed and the amount of water drunk (Table 2).

The essential point is that, with meat eating, less water was required than with crusts. With fractional true feeding, when no water was given, less crusts were eaten than when water was supplied. Thus, Zhuchka ate 110 g in experiment No. 4, and 270 g in experiment No. 14. The average amount of crusts consumed in the nine experiments in which drink was supplied exceeded 320 g.

The absence of drink did not affect the amount of meat consumed: in experiment No. 12, when no fluid was given, Zhuchka consumed 1,240 g of meat, and the mean amount of meat eaten in seven experiments in which fluid was given was slightly greater than 1,100 g.

TABLE 2. Drinking Reaction in Dogs in Fractional True and Sham Feeding (Meat)

Number of Experiment	Name of dog	Amount of food consumed (in g)	Time of feeding until refusal of food (in minutes)	Feeding reaction	Drinking reaction
		Fractional true feeding with drink freely offered			
7	Zhuchka	960	155	No refusal	Drank 650 ml
9	"	1170	190	" "	" 650 "
10	"	960	175	Refusal	" 400 "
12*	"	900	145	No refusal	" 250 "
18	"	1200	195	" "	" 450 "
19	"	1320	215	Refusal	" 200 "
4	Mal'chik	1170	200	"	" 1000 "
1	Belyi	1140	185	"	" 200 "
3	"	960	105	"	" 350 "
		Fractional true feeding without drink			
15	Zhuchka	1240	195	No refusal	
		Fractional sham feeding while drink freely available			
5**	Mal'chik	1140	185	No refusal	Drank (amount not recorded)
14	"	1110	180	" "	Did not drink
15	"	1140	185	" "	" "
16	"	1650	275	" "	" "
17	"	1140	180	" "	" "
19	"	2150	360	" "	" "

* Portion of meat weighed 50 g and not 30 g.

** The experiment was performed before esophagotomy, and there was an open gastric fistula.

Interesting results were obtained in experiments with fractional sham feeding. It was only in the first experiments with crusts (No. 6) and with meat (No. 5, performed before esophagotomy and with an open gastric fistula), and in the single experiment (No. 18) with bread, that Mal'chik showed a weakly positive drinking reaction. In all the other experiments with bread and meat, not only did the dog drink none of the water which was on the stand, but actually turned away from it when it was brought up to its mouth.

Thus, with fractional sham feeding with either crusts or meat, no positive drinking reaction occurred, although it is well shown with fractional true feeding. It may therefore be concluded that the latter effect is due to the action of food on the gastric receptors.

When giving fractional sham feeds to an esophagotomized dog, when the food does not act on the gastric receptors, the animal is not stimulated to drink. In experiment No. 5 on the dog Mal'chik, in whom a gastric fistula was established before esophagotomy, when the dog was supplied with meat, i. e., when the food exerted some action on the gastric receptors, a positive drinking reaction was induced. In this experiment, the feeding was only partially sham, because the food was not returned through the gastric fistula until 40 minutes after the animal had begun to eat. During this time the gastric receptors were exposed to the meat, so that a positive drinking reaction was induced.

The significance of the reflex excitation of the drinking center through the action of the food on the gastric receptors is probably that it regulates water balance after it has been disturbed through the abundant secretion of digestive juices during the digestion of the food.

The gastric receptors concerned are probably osmoreceptors. Borshchevskaya [3] showed that osmoreceptors are present in the digestive tract, and I. N. Zhuravlev [4,5] has studied the effect on drinking behavior of different concentrations of salt introduced into the stomach.

SUMMARY

A study was made of drinking behavior in dogs during fractional true and fractional sham feeding with dried crusts or cooked meat. When the food was consumed normally, they drank shortly after swallowing the first portions of food, and continued to drink during the whole time that they were eating. When sham-fed, they did not drink. The stimulus to drink is therefore evoked by the action of food on the gastric receptors.

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CHANGE IN THE SORPTIVE PROPERTIES OF A MYOCARDIUM AFFECTED BY ADRENALIN

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Translated from Byulleten'Éksperimental'noi Biologii i Meditsiny, Vol. 52, No. 7, pp. 33-38, July, 1961
Original article submitted June 2, 1961

The fact that large doses of adrenalin have a toxic effect on the heart of warm-blooded animals is widely acknowledged in the literature. The morphological picture of this type of myocarditis has been described in detail by several researchers [1,4].

In earlier investigations [2,3], we found that a single intramuscular injection of a large dose of adrenalin (0.5-0.8 ml of a 1 :1000 solution) administered to rats causes destructive changes to develop in the myocardium. EKG changes indicating disturbance of myocardial trophicity were observed even during the first few hours after the injection: the voltage and form of the P, R, S and T waves changed, and the ST interval shifted away from the isoelectric line. Microscopic examination of fixed histological preparations obtained 3-12 hr after the adrenalin injection disclosed swelling and homogenization of individual muscle fibers, while preparations obtained after 18 hours or more showed disintegration of certain groups of muscle fibers and a sharply pronounced cellular reaction, both of which usually became more marked by the end of the second day. Later, connective tissue appeared, and the foci of muscle fiber disintegration were replaced by scar tissue. The destructive changes were concentrated primarily in the papillary muscle, the wall of the left ventricle and the interventricular septum.

By means of electrocardiography and microscopy of fixed histological preparations, therefore, we were able to trace the course of the pathologic process induced in the myocardium by the action of toxic doses of adrenalin. By these means, however, the myocardial injury can only be examined at the later stages of its development, as these methods can reveal nothing of the condition of the myocardium during the first hours following the action of harmful doses of adrenalin. In order to determine this aspect of adrenalin's effect, we resorted to the method of intravital staining developed by D. N. Nasonov. This method not only makes it possible to qualitatively demonstrate injury to cell structures resulting from the action of stimulants, but also permits quantitative analysis of such injury according to the change in sorption of a stain by the tissue [5,6].

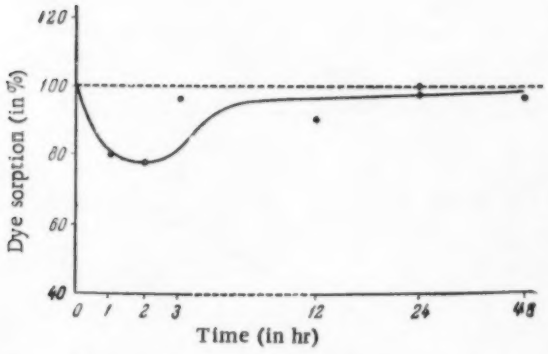
METHOD

The experiments were performed on rats weighing 180-250 g each. Adrenalin was injected intramuscularly in a dose of 0.5 ml of a 1: 1000 solution. The animals were sacrificed by decapitation after 1,2,3,12,24 and 48 hours. The heart was taken out of the thoracic cavity and washed out with a Ringer's solution administered through a cannula inserted in the aorta, after which 10 ml of a 0.05% solution of neutral red stain prepared in a Ringer's solution without soda was passed through this same cannula for five minutes. When the cavities of the heart and vessels had been washed out and filled with the stain solution, the heart was placed in a small glass containing the stain solution for a period of 20 min.

At the end of this period, it was taken out of the dye solution and washed in a Ringer's solution; then two transverse incisions were made through the whole thickness of the myocardium at the level of the papillary muscles, 1-2 mm apart. The resulting myocardial section was put into acidified ethyl alcohol in order to extract the stain. The alcohol extracts were measured photometrically with an electrophotocolorimeter. The amount of stain absorbed was determined in arbitrary units of extinction × 1000 per 1 mg dry weight of the heart muscle, or expressed in percent of the control coloration, which was taken as 100%. The hearts of all the experimental and control animals were examined under the microscope. The Student-Fisher method of statistical processing was used to check the

TABLE 1. Change in Myocardial Sorption of Neutral Red Attending Adrenalin Myocarditis

Animal group	Number of Animals	Time (in hr) after adrenalin injection	Dye sorption in conventional units: $\frac{\text{extinction}}{\text{weight}} \times 1000$ (M± m)	α	Dye sorption (in % of control)
Experimental	8	1	5.6±0.49	0.968	80.0
Control	8	-	7.0±0.46		
Experimental	8	2	5.5±0.23	0.995	78.5
Control	8	-	7.0±0.46		
Experimental	8	3	4.9±0.80	0.721	96.0
Control	8	-	5.0±0.28		
Experimental	8	12	4.0±0.96	0.832	90.7
Control	9	-	5.4±0.33		
Experimental	8	24	5.0±0.25	0.500	100.0
Control	8	-	5.0±0.28		
Experimental	8	48	5.3±0.41	0.578	98.1
Control	9	-	5.4±0.33		



Curve of myocardial sorption of neutral red during adrenalin myocarditis.

significance of the results obtained. When the difference probability α was at least 0.950, the results were assumed to be significant.

RESULTS

The experiments showed a considerable decrease in the sorption of the stain by the myocardial tissues during the first few hours after the adrenalin injection. Sorption was 80 and 78.5% of the control, for example, after 1 and 2 hr respectively. These results proved to be statistically significant. At the later stages (3-48 hr), sorption was somewhat less in the experimental animals than in the control, but this difference was not proven statistically significant (Table 1, see figure also).

Microscopy of the hearts of the control animals showed clearly striated muscle fibers containing single granules. The background was diffusely stained. Occasional (1-2 per preparation) nuclei could be seen. Vital staining of the hearts affected by adrenalin did not demonstrate any substantial changes in the muscle fibers. Predominantly lengthwise striation was only observed in a few cases. Besides this, a great quantity of connective tissue cells and formed white blood elements containing many granules of the dye was found in all the animals killed 1 and 2 days after the administration of adrenalin. They corresponded to the accumulations of cellular elements observed on the fixed histological preparations prepared at the same stages in the disease. However, neither a more intense background stain nor any muscle cell nuclei were observed in any case. This was an unexpected development, since the dynamics of the subject affection had been traced on histological preparations from the slight changes which occurred in the early stages to the disintegration of the muscle fibers and sharply pronounced cellular reaction evident at the later stages.

We were unable to find any information in the literature in regard to vital staining of the heart muscle of warm-blooded animals under normal conditions and after the infliction of injury. It could then be proposed that, in the first place, the myocardium of warm-blooded animals has its own specific characteristics and does not respond to injury by the manifestation of nuclear structures or increased sorption of dye and, in the second, that the failure of the sorption of neutral red to increase was due to change in the acid-alkali balance in the myocardial tissue attending the action of adrenalin.

TABLE 2. Effect of Hypotonic Medium on Myocardial Sorption of Neutral Red

Animal group	Number of Animals	Experimental conditions	Dye sorption in conventional units: $\frac{\text{extinction}}{\text{weight}} \times 1000$ (M± m)	α	Dye sorption (in % of control)
Experimental	7	Stain prepared in a four times diluted Ringer's solution	18.4± 3.23	0.998	279.0
Control	7	Stained in isotonic solution	6.6± 0.67		
Experimental	5	Stained in isotonic solution after removal of heart from hypotonic medium	7.2± 0.46		
Control	8	Stained in isotonic solution	5.5± 0.42	0.991	130.9

TABLE 3. Phenol Red Staining of Myocardium in Adrenalin Myocarditis

Animal group	Number of Animals	Time after adrenalin injection (in hours)	Dye sorption in conventional units: $\frac{\text{extinction}}{\text{weight}} \times 1000$ (M± m)	α	Dye sorption (in % of control)
Experimental	5	1	4.2± 0.37	0.578	102.0
Control	6	-	4.1± 0.18		
Experimental	4	2	4.6± 0.15	0.613	97.8
Control	4	-	4.7± 0.31		
Experimental	8	24	4.9± 0.22	0.721	104.2
Control	8	-	4.7± 0.22		
Experimental	5	48	4.1± 0.36	0.850	89.3
Control	5	-	4.6± 0.29		

TABLE 4. Effect of High Temperature on Myocardial Sorption of Phenol Red

Animal group	Number of Animals	Dye sorption in conventional units: $\frac{\text{extinction}}{\text{weight}} \times 1000$ (M± m)	α	Dye sorption (in % of control)
Experimental	3	24.1± 3.75	0.999	515
Control	8	4.7± 0.22		

To prove the first hypothesis, we performed experiments on rats' isolated hearts treated with a hypotonic medium. In the first series of experiments, the heart was first washed free of blood, then stained as described above except that the stain was prepared in a four times diluted Ringer's solution.

The experiments showed a sharp increase in sorption of the stain under these conditions, to 279% of the control, which was regarded as 100% (Table 2). Microscopic examinations of these hearts demonstrated a large number of nuclei in the muscle fibers.

The extent to which the stain was sorbed by the tissue in these experiments could be affected by the conditions

of hypotonia under which the heart muscle was stained. In the second series of experiments, therefore, the heart was stained in a stain solution prepared by the usual method, and myocardial injury was induced by placing the heart muscle for 20 min in a Ringer's solution diluted four times. Under these conditions, myocardial sorption of the stain was 130% (see Table 2), and microscopic examination disclosed well-defined nuclear forms.

In both these cases, therefore, the isolated heart responded to injury by a typical paranecrotic reaction: the appearance of nuclear structures and increased dye sorption. The symptoms of paranecrosis were considerably more pronounced in the first series of experiments than in the second. This was probably due to the fact that the paranecrotic symptoms (or "hypotonic numbing" of muscular structures) are reversible for a certain period, and the isotonic conditions under which the heart was stained considerably reduced these symptoms in the latter experiments.

A series of experiments in which the acid stain phenol red was used was performed in order to prove the second hypothesis–the effect of possible change in the pH of the medium on the fixation of neutral red. The pH is known to become more acid under conditions of inflammation. Therefore, if this holds true in adrenalin myocarditis, no increase in dye sorption would occur even in the presence of injury to the cellular structures if the dye used were the basic stain neutral red. Acid dyes, on the other hand, would produce a more intense stain.

The experimental procedure in this case was the same as that described above. Staining was done with a 0.03% solution of phenol red, prepared in a Ringer's solution. The staining time was 20 min. The experimental animals were examined 1,2,24 and 48 hr after the adrenalin injection (Table 3).

As Table 3 indicates, these experiments showed no increase in dye sorption either during the first two hours after the injury was inflicted or at the later stages (after 1-2 days), when the destructive changes in the myocardial tissue had become clearly apparent.

Dye sorption increased by more than five times when the isolated heart was injured by high temperatures (immersion for 5 min in a Ringer's solution at a temperature of 60° C). In this case, therefore, the isolated heart responded to injury by a typical paranecrotic reaction–increased dye sorption (Table 4).

The use of the vital staining method to study experimental adrenalin myocarditis disclosed considerable decrease in the sorption of the basic stain neutral red during the first few hours after the administration of adrenalin. This could be an early objective sign of the changes which occur in the protoplasm of the myocardial muscle cells during the initial stages of the myocardium's reaction to toxic doses of adrenalin. D. N. Nasonov and his co-workers [5] often observed the intravital stainability of the protoplasm to decrease under various influences. No exact explanation of this phenomenon has yet been offered. It could be a defense reaction of the tissue to irritation, increasing its resistance to various harmful agents [7].

In the later stages of the process of injury, however, we did not observe the increase in dye sorption which usually occurs in animals under certain influences. Perhaps, under conditions of an intact organism, reparative processes to heal the injury develop in response to the latter, and this effects dye sorption.

The lack of increase in the stainability of live myocardial cells could also be due to the fact that the injury induced by adrenalin did not encompass the entire muscle, as occurred, for example, in our experiments with the action of hypotonic solutions and high temperature on the heart, but affected only certain portions of it, and increased sorption by these small portions may not be reflected by the over-all result. However, the fact that microscopic examination of the sections never disclosed the appearance of nuclei, which is one of the first signs of injury to the cell protoplasm–despite the presence of cellular infiltration, and therefore, muscle fiber disintegration–would seem to negate the latter hypothesis.

Nor can the lack of increase in the stainability of the myocardial tissue under conditions of adrenalin myocarditis be ascribed to a shift of the pH of the medium toward the acid side, as is usually observed in inflammation, since the sorptive properties of the myocardium showed no change when acid dyes were used.

The experimental data obtained, therefore, permits the hypothesis that the failure of the stainability of the myocardial tissue to increase in adrenalin myocarditis under conditions of an intact organism is due to reparative processes which develop to heal the injury.

SUMMARY

A method of vital staining was used to investigate the sorptive property of rat cardiac muscle affected by adrenalin.

In 1-2 hours after injecting 0.5 ml of adrenalin solution (1: 1,000) intramuscularly the sorption of neutral red shows a marked reduction, whereas in 12-24 hours it does not differ materially from the control. The absence of increased vital staining of myocardial tissues, notwithstanding the presence of destructive changes in them (detectable by histological examination), may be explained by repair processes developing in the intact organism in response to injury.

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DISTRIBUTION IN THE ORGANS AND TISSUES OF INTRAVENOUSLY ADMINISTERED RADIOACTIVE PHOSPHORUS IN INFECTIOUS PERITONITIS

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 52, No. 7,
pp. 38-40, July, 1961
Original article submitted May 21, 1960

Peritonitis is one of the most serious diseases of the organs of the abdominal cavity. According to the data of different authors [4,16,19] the lethality in acute diffuse peritonitis is 4-10%.

According to the data of I. I. Deryabin [6] and K. S. Simonyan [17], all forms of metabolism are affected quickly and severely in peritonitis, which is probably a result of change in the permeability of the cell membranes. N. V. Okunev [14] notes that almost every nosological unit is based on some permeability disorder.

The many works carried out in N. N. Zaiko's laboratory [8] have given reason to conclude that the permeability of cell membranes is of great importance in protection of an organ from the action of toxins. K. F. Dogaeva [7] used the method of intravital microscopy to establish that the capillary walls of different organs and tissues are variably permeable by stains, the permeability depending primarily on both the structure of the capillary endothelium and the functional condition of the organ. The works of several authors[1,5,10,15,18] have demonstrated that the functional condition of almost every organ changes in peritonitis.

This study of the permeability of the vessels of the internal organs in infectious peritonitis was based on the above data.

METHOD

The work was performed on 20 rabbits and consisted of two series of experiments. In the first series, performed on ten animals, we studied the permeability of the capillaries in the internal organs under conditions of infectious peritonitis. In the second series, we studied capillary permeability in these same organs in ten healthy rabbits. This series served as the control.

Laparotomy was performed on the animals of the first series under local anesthesia (10-15 cc of a 0.5% Novocain solution subcutaneously administered) after preparation of the operative area. Then the stomach was perforated with a red-hot rod 0.3 cm in diameter. The cauterizing action of the rod created a zone of necrosis and a gaping perforation. Through the perforation, the gastric contents flowed out into the abdominal cavity. A 24 hour culture of virulent Staphylococcus aureus screened from purulent exudate obtained from sick people was then introduced into the gastric lumen through the perforation in a dose of one billion microbes. The subcutaneous administration of this microbe emulsion to a rabbit induced necrosis and purulent inflammation of the cellular tissue, while its intravenous administration caused death. The wound in the abdominal cavity was tightly sutured (by a method borrowed from I. I. Neimark [12]). In all the animals of this group, the development of peritonitis was indicated within 24 hr by the presence of exudate in the abdominal cavity, rise of body temperature and increased number of leukocytes in the peripheral blood.

We used the radioactive phosphorus isotope P^{32} ($Na_2HP^{32}O_4$) to determine vascular permeability. Twenty-four hours after the operation described, radioactive phosphorus was injected into the marginal vein of the ear of the experimental and of the control (unoperated) animals in a dose of 22 μ C per 1 kg weight. One hour after the phosphorus was administered, the animals were sacrificed by air embolism, and specimens were prepared from the internal organs to determine capillary permeability.

The following organs were examined: the parietal and visceral peritoneum, skin, lungs, diaphragm, heart, omentum, liver, spleen, kidney, adrenal gland and brain. A total of 140 specimens were prepared. The permeability of the capillaries in these organs was determined by I. A. Mukhamedzhanov's method [11]. Pastes prepared from each organ were applied in amounts of 200 mg to pieces of filter paper of equal weight and area. The pieces of paper with the tissue were dried somewhat, then examined for radioactivity on a type B counting apparatus with an MS-4 electronic counter. The number of impulses per min was computed after deduction of the background, and average figures were computed from three determinations. This method made it possible to judge the general capillary permeability [13].

RESULTS

Radioactivity of Tissues of Various Organs in Control Rabbits (without Peritonitis) and Animals with Peritonitis. Number of Impulses (in %) Are Averages of Ten Determinations

Experimental organ	Control rabbits		Rabbits with peritonitis		Average difference (%)
	Impulses	Mean square deviation and average error	Impulses	Mean square deviation and average error	
Brain	100	$\sigma \pm 4.00$ $m \pm 1.02$	140	$\sigma \pm 3.74$ $m \pm 1.01$	+40
Skin	160	$\sigma \pm 4.80$ $m \pm 1.05$	200	$\sigma \pm 5.29$ $m \pm 1.07$	+40
Lung	200	$\sigma \pm 5.29$ $m \pm 1.06$	220	$\sigma \pm 5.74$ $m \pm 1.08$	+20
Heart	200	$\sigma \pm 5.92$ $m \pm 1.08$	220	$\sigma \pm 4.80$ $m \pm 1.05$	+20
Liver	200	$\sigma \pm 4.80$ $m \pm 1.05$	300	$\sigma \pm 5.83$ $m \pm 1.08$	+100
Spleen	200	$\sigma \pm 3.00$ $m \pm 0.94$	220	$\sigma \pm 5.57$ $m \pm 1.07$	+20
Omentum	140	$\sigma \pm 3.61$ $m \pm 1.01$	160	$\sigma \pm 2.83$ $m \pm 0.89$	+20
Visceral peritoneum	200	$\sigma \pm 7.48$ $m \pm 2.03$	280	$\sigma \pm 5.10$ $m \pm 1.06$	+80
Parietal peritoneum	140	$\sigma \pm 4.90$ $m \pm 1.05$	220	$\sigma \pm 5.00$ $m \pm 1.05$	+80
Diaphragm	140	$\sigma \pm 5.10$ $m \pm 1.06$	240	$\sigma \pm 5.83$ $m \pm 1.08$	+100
Kidney	160	$\sigma \pm 3.87$ $m \pm 1.02$	240	$\sigma \pm 4.58$ $m \pm 1.04$	+80
Adrenal gland	180	$\sigma \pm 4.80$ $m \pm 1.05$	200	$\sigma \pm 5.48$ $m \pm 1.07$	+20

The results obtained are given in the table.

It is evident from the data presented in the table that, as Starling has indicated [21], capillary permeability is not stable in healthy animals. The variations in permeability which occur under normal physiologic conditions are evidently connected with the course of the metabolic processes.

The results obtained indicate that the vessels of all the internal organs of the rabbits with peritonitis showed considerably greater permeability than those of the control animals. In such organs as the lungs, heart, spleen, omentum, and adrenal gland the vessels' permeability was 20% higher, and in the skin and brain vessels 40% higher, while the vessels of the parietal and visceral peritoneum were 80% more permeable than those of the controls. The number of impulses found in the liver and diaphragm specimens showed a 100% increase in permeability as compared with the controls.

The data we obtained, therefore, indicate that the properties of the whole capillary network are disturbed in peritonitis, apparently as a result of metabolic disturbance and the accumulation of toxic products which act on the vascular walls. The increased permeability of the vessels of the internal organs creates favorable conditions for the absorption of the pathologic metabolic products which form in great quantity under conditions of an inflammatory process as extensive as peritonitis [2, 3, 9, 20]. On the basis of these data, it is easy to explain the symptoms of meningoencephalitis observed by Kh. G. Gafurov [5] in animals with experimental purulent peritonitis.

SUMMARY

Changes in the capillary permeability of internal organs, skin and brain occurring during infectious acute peritonitis were studied with the aid of radioactive isotopes. The results obtained have demonstrated an increased capillary permeability in all the internal organs, skin and brain. The greatest rise of capillary permeability was noted in the organs directly adjacent to the inflammation focus, or in those participating in the neutralization and excretion of toxic substances from the body.

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THE EFFECT OF BENZENE ON THE LEUKO-AGGLUTINATING AND RETICULOCYTOGENIC PROPERTIES OF SERA

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 52, No. 7, pp. 40-44, July, 1961
Original article submitted July 8, 1960

Investigators and clinicians have studied the question of benzene's effect on the composition of the blood over a period of many years [1,8,11,12].

The action mechanism of benzene cannot be considered fully understood, however, since most authors mention only the direct effect of this substance on the bone marrow, and only the works of P. Ya. Mytnik [10] mention the important part played by its action on the sympathetic nervous system in the development of benzene leukopenia. In 1958, L. S. Musikhin found that the osmotic resistance of leukocytes changed in a series of cases under conditions of experimental benzene poisoning [9]. One would expect, by analogy with other types of toxic injury to the blood system, that the leuko-agglutinin type of auto-antibodies would appear in the serum of people and animals poisoned with benzene.

Another advantage of the experimental model of benzene poisoning is that it can be used to trace the reticulocytogenic and erythropoietic properties of blood sera in hypoplastic and aplastic conditions of hemopoiesis.

A similar type of investigation showed that massive x-ray irradiation does not affect hemopoietine [erythropoietic substance found in circulating blood] production [13]. We formed analogous conclusions in our clinical observations on patients with aplastic anemia (1959).

With the above data in mind, we studied both the changes in blood composition caused by poisoning with different doses of benzene and the changes in the leukoagglutinating and reticulocytogenic properties of the poisoned animals' sera, taking into account the character of the poisoning.

METHODS AND RESULTS

Change in the Composition of the Blood Attending Benzene Poisoning. We poisoned a total of 27 rabbits, which we separated into three groups. The ten rabbits of the first group received 0.3 ml benzene per 1 kg weight daily for five days; the ten rabbits of the second group were each injected with 1 ml benzene per 1 kg weight until clearly expressed aleukia developed and the animal died (injections given for 10-12 days), and the seven rabbits of the third group were each injected with 1 ml benzene per 1 kg weight every other day for 10-12 days until the appearance of pronounced changes in the blood composition.

Benzene was dissolved in an equal volume of peach oil and injected subcutaneously. The composition of the blood (erythrocytes, hemoglobin, reticulocytes, leukocytes and leukocyte formula) was studied for two weeks before poisoning, during poisoning and for two weeks after the daily administration of benzene ceased.

The changes in blood composition attending benzene poisoning depended on the dose. For example, in all ten of the rabbits which received the 0.3 ml benzene per 1 kg weight dose (a total of 5-8 g benzene), leukopenia developed on the 5th-7th day (average number of leukocytes constituted 44% of the original); the absolute content of leukocytes varied between 1800 and 6000 per 1 mm^3. In eight cases, erythrocytopenia was also observed (an average decrease of 950,000 per 1 mm^3). The hemoglobin content decreased by 12 units. Reticulocytopenia was also observed in every case (reticulocytes decreased to 30% of original level).

Fig. 1. Change in blood composition attending benzene poisoning: I) original level, II) first group of poisoned rabbits (0.3 ml benzene per 1 kg animal weight daily), III) second group (1 ml benzene per 1 kg weight daily), IV) third group (1 ml benzene per 1 kg weight every other day); a) erythrocytes, b) hemoglobin, c) leukocytes, d) reticulocytes.

The changes observed the 7th day in the blood composition of the animals of the second group (benzene administered in doses of 1 ml per 1 kg weight) were more pronounced. For example, the number of leukocytes constituted only 14% of the original, and the absolute content varied between 200 and 1000 per 1 mm^3 (the original figures being 9800-12,100 per 1 mm^3). Reticulocytopenia was also more pronounced: the reticulocytes constituted 8% of the original level and, in six cases, had totally disappeared from the blood. At the same time, the changes observed in the number of erythrocytes were about the same as those observed in the first group of rabbits (an average decrease of 980,000 per 1 mm^3), while the hemoglobin decreased 10 units. The changes in the leukocyte formula consisted in lymphopenia and relative neutrophilia (pseudo-eosinophilia).

Leukopenia was recorded in six out of seven cases in the experiments on the third group of animals (1 ml benzene per 1 kg weight injected every other day); the leukocytes decreased to 21% of the original number on the day of the last injection. The reticulocyte content also decreased in six out of seven animals to an average 16% of the original. Anemia was more pronounced; the number of erythrocytes decreased an average of 1,500,000 per 1 mm^3, while the hemoglobin decreased an average of 15 units.

These changes are shown in Fig. 1.

Benzene administration, therefore, not only causes clearly expressed leukopenia, but disturbs erythropoiesis too, as L. S. Musikhin [9] also observed.

Comparison of Number of Leukocytes and Leuko-Agglutination Reaction in Benzene Poisoning

Type of reaction	Number of leukocytes per 1 mm^3		
	Up to 3,500	3,600-4,500	Over 4,500
Positive	12	0	0
Negative	0	3	2

Change in the Leuko-Agglutinating Properties of the Serum. Although various types of leuko-agglutinins have been discovered in many diseases of the blood system associated with leukopenia, this question has as yet received little study in relation to benzene poisoning.

The rabbits' serum was examined for leuko-agglutinating properties before the animals were poisoned and on the last day of the benzene administration. The leuko-agglutination reaction was done according to the usual method. A leukocyte suspension was prepared from the blood of healthy rabbits; 0.4 ml of the experimental serum was then added to 0.2 ml of this suspension, and the mixture was incubated at 38°C for an hour, after which smears were prepared from it. The leuko-agglutination reaction was evaluated immediately, first without and then with staining according to May-Grunwald. The intensity of leuko-agglutination was rated as +, ++ and +++.

Before the poisoning, a weakly positive reaction (+) was observed in only 1 out of 17 healthy rabbits. The reaction became uniformly positive in the animals poisoned with benzene in the dose of 0.3 ml per 1 kg weight, sharply so in the six out of ten animals in which the leukocytes numbered less than 3,500 per 1 mm^3 blood. When

the reaction was conducted with seven rabbits which had received the 1 ml dose of benzene per 1 kg weight, it was positive in every case. In this group of poisoned animals, the leukocytes numbered 2,500 or less per 1 mm^3 (see table).

Therefore, the leukopenia caused by the administration of benzene can, in certain cases, especially in the case of low leukocyte content, be associated with the appearance of the leuko-agglutinin type of antibody as well as with the direct effect of benzene on hemopoiesis.

Study of the Reticulocytogenic Properties of the Sera. As a test of hemopoietic activity, rabbit's serum obtained after coagulation of the blood and clot retraction was subcutaneously injected after 24 hours in a dose of 3 ml into two healthy rabbits, the blood composition of which was studied for 14 days before the injection and 14 days afterwards.

Only two out of the ten sera obtained from healthy animals caused the number of reticulocytes in the rabbits into which they were injected to double and triple on the second and third day after the injections (2.5% and 1.3% to 5% and 4.2% respectively). The variations observed in the other eight cases did not exceed those observed before the experiment. Contrary to the results observed with the administration of human serum, the erythrocyte content increased an average of 558,000 per 1 mm^3 in five cases, while the variations observed in the other five cases did not exceed the normal. Therefore, the serum of healthy rabbits can cause erythrocytosis and reticulocytosis, but more frequently does not possess such properties (Fig. 2). The changes observed persisted for two to three days.

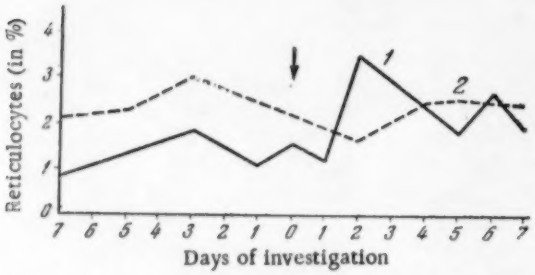

Fig. 2. Change in the reticulocyte content in rabbits following administration of 3 ml of serum from 2 healthy animals (1,2). The arrow shows the day the serum was administered.

The sera of rabbits poisoned with benzene were examined for reticulocytogenic properties on the last day the poison was administered. The 27 experimental sera were injected into 54 healthy rabbits. In the 17 animals of the less severely poisoned first and third groups, these properties were approximately uniform.

As compared with the serum from the healthy rabbits, the serum from the poisoned rabbits did not, in 6 out of 17 cases, cause fluctuations in the reticulocyte content exceeding those observed in the control experiments (an increase from the original 1-1.9% to 2.8-3.3% on the second to fourth day after administration). The hemoglobin content did not change substantially (varying 8 units at most). At the same time, the number of erythrocytes decreased an average of 930,000 per 1 mm^3 in 11 out of 17 cases. We could observe no correlation between the reticulocytogenic and anematizing properties of the sera.

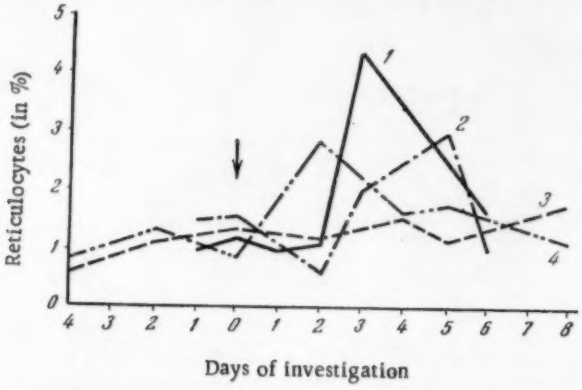

Fig. 3. Change in the reticulocyte content in rabbits following administration of 3 ml of serum from 4 animals poisoned with benzene (1,2,3,4). The arrow shows the day the serum was administered.

Nor was the original content of reticulocytes, hemoglobin and erythrocytes in the blood of the animal donors of any particular significance. As the results of the third group of experiments show, however, the serum from the most severely poisoned animals caused acute reticulocytosis in four out of seven cases (Fig. 3). For example, marked reticulocytosis (an increase of almost 4 $\frac{1}{2}$ times) was induced by serum taken from rabbit No. 23 when the leukocyte content in this animal constituted a total of only 600 per 1 mm^3 as compared with the original 10,400, the reticulocyte content was 0.1% compared with the original 2.8% and the hemoglobin was 60 units as compared with the original 80. However, the sera of rabbits Nos. 39 and 40, in which the composition of the blood was almost analogous to that of No. 23, did not exhibit such properties.

Therefore, benzene poisoning, even when so acute as to cause the animal's death, does not eliminate the ability of the sera to cause reticulocytosis when administered to healthy rabbits; on the contrary, these properties are more frequently observed, perhaps due to the formation in the serum of substances promoting erythrocyte destruction. Specific proof is required, however, before this hypothesis can be considered conclusive.

SUMMARY

Experimental benzol poisoning of rabbits (in the doses of 0.3 to 1 ml per kg of body weight) provokes, apart from the reduction of the leukocyte count, a marked reticulocytopenia. The appearance of antibody leukoagglutinins, which form at the time of the benzol action, may play a certain role in the mechanism of the effect of the latter on the leukocyte portion of the blood. The serum of healthy animals upon administration provoked reticulocytosis in 2 out of 10 healthy animals. The serum of the benzol-poisoned rabbits (especially in severe cases of poisoning) not only lost these properties, but even provoked reticulocytosis in a greater percentage of cases, in 15 of the 27).

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THE INFLUENCE OF THE NERVOUS SYSTEM ON BONE MARROW HEMOPOIESIS

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(Presented by Academician V. N. Chernigovskii)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 52, No. 7,
pp. 45-48, July, 1961
Original article submitted October 10, 1960

Carrying out an idea of I. P. Pavlov's, Academician K. M. Bykov conducted research on the functional interrelations between the cerebral cortex and the internal organs [2]. Later, V. N. Chernigovskii and A. Ya. Yaroshevskii [7] established experimentally that the hemopoietic organs possess a well-developed innervation, modification of which can cause marked changes in hemopoiesis.

In explanation of this, V. N. Chernigovskii and A. Ya. Yaroshevskii [7] established that there is a direct relationship between the functional condition of the cerebral cortex and the composition of the blood. The data obtained by these authors have been confirmed by other researchers.

Data indicating central regulation of hemopoiesis have also been obtained by N. A. Grigorovich [3] experimentally and V. A. Beier [1] on clinical material.

Starting with Academician A. D. Speranskii's hypothesis that a previously existing pathologic process can be reproduced by inflicting trauma on the nervous system, A. Yu. Tilis and Kh. Z. Lyubetskii [5], using the "second stroke" method, discovered trace phenomena during the regeneration of red blood in animals with saturnine anemia. A. Yu. Tilis and G. A. Suleimanova [6] et al. demonstrated the dependency of the leukocytic reaction on the functional condition of the autonomic nervous system. The literature, then, contains sufficient data to indicate that the nervous system "is the regulator rightfully regulating the whole blood system" (K. M. Bykov [2]).

TABLE 1. Change in Peripheral Blood Composition in Healthy Animals after "Collision"

Time examined	Hemoglobin content (in %)	Number of erythrocytes	Number of leukocytes
Before collision	78	5 880 000	11 790
30 min after collision	73	5 786 000	13 616
2 hr " "	71	5 681 000	16 667
24 hr " "	76	5 408 000	14 816
48 hr " "	78	5 621 000	13 533
96 hr " "	78	5 878 000	14 883

In earlier investigations [4], we were able by means of the "towing" technique to reproduce to a certain extent a peripheral blood picture, the specific hematologic indices of which suggested the blood picture observed after phenylhydrazine poisoning.

The purpose of this work was to determine the influence of the nervous system on bone marrow hemopoiesis and on the composition of the cellular element of bone marrow in hemotoxic anemia under experimental conditions.

We wished to determine the nature of the hemopoietic changes occurring in healthy animals under conditions of higher nervous activity collapse ("collision") and to detect by this method the trace reactions of bone marrow hemopoiesis on a background of preliminary phenylhydrazine poisoning.

METHOD

Twelve dogs were used in the experiments. The initial pathologic process was induced by administering 0.5-1 ml of a 2% phenylhydrazine hydrochloride solution per kg weight to the animal until a pronounced picture of anemia had developed.

When the normal composition of the blood had been restored, higher nervous activity collapse was induced in the animal by acute intensification of the excitation processes. To this end, painful stimulation by 10 volt alternating current was applied to the animal the moment it touched food. This caused collision of the excitation process induced by the offer of food with the defense reflex associated with painful stimulation.

The composition of the formedelements in the bone marrow and peripheral blood was studied before the experiment, after phenylhydrazine poisoning, during restoration of the hematologic indices and after the "collision". Control experiments were conducted in which the painful stimulation by electric current was not administered in conjunction with food. In these experiments, either the hematologic indices showed no apparent change or some slight increase in the number of erythrocytes was observed.

RESULTS

After the "collision", we were able to establish that collapse of higher nervous activity is attended by some change in hemopoiesis in healthy dogs. In the bone marrow, for example, the young, undifferentiated cells (hemohistioblasts, hemocytoblasts, myeloblasts, et al.) increased from an average 2.23% to 13.23%.

The bone marrow neutrophil index, computed according to the formula $\frac{PRM+m+y}{s+p}$*, increased from 0.44 to 0.47, which indicates intensified hemopoiesis of the myelocytic precursors of neutrophils. Because of this shift to the left, the number of mature neutrophils diminished. There was some increase in the relative content of eosinophils and red bone marrow elements (which increased respectively from 3.16 to 3.66% and from 36.9 to 40.3%) after the "collision".

The result obtained shows that "electrocollision" causes some intensification of the regenerative processes, especially with respect to the white elements of the bone marrow.

The peripheral blood indices are given in Table 1.

Even 30 min after the collision, the number of leukocytes had increased in a majority of cases; this index continued to increase for 2 hr until it was an average of 1.5 times higher than the original. It then decreased slightly, but remained higher than the original even after two to three days.

A slight decrease in the hemoglobin content and number of erythrocytes occurred immediately after the "collision."

The data presented were compared with the results of the second cardinal series of experiments in which the hematologic indices were studied after "collision" on a background of preliminary phenylhydrazine poisoning (Table 2).

A picture of macrocytic hemolytic anemia usually developed in the animals poisoned with phenylhydrazine. The ratio of the myeloblastic elements to the erythroblastic ($\frac{\text{leukocytes}}{\text{erythrocytes}}$), by which the erythropoietic function of the bone marrow is usually evaluated, was 2.2 : 1 in our experiments before the poisoning. This ratio changed to 1:2.5 during the development of anemia following phenylhydrazine poisoning.

If the "collision" was reproduced during the period when the original hemopoietic indices were restored, this ratio again decreased to the level typical of the anemic condition. The erythroblastic elements themselves followed a similar pattern. After phenylhydrazine poisoning, for example, the number of basophilic and polychromatophilic normoblasts was 1.85 times higher than the original level, and when "electrocollision" was induced after restoration of the hematologic indices, the number of these cellular elements increased to the same degree (1.8 times) as after phenylhydrazine poisoning. The number of orthochromic normoblasts was halved in both cases.

From the data presented, it is evident that "collision", on a background of previous phenylhydrazine poisoning, causes changes in the bone marrow hemopoiesis of animals, attended by intensification of the processes of erythroblast maturation. Finally, the changes which develop in the blood system recall the hemopoietic changes observed

* PRM - promyelocytes, m - myelocytes, y - young, s - stab, p - polymorphonuclear.

TABLE 2. Hemopoiesis in Hemolytic Anemia under Conditions of "Electrocollision"

Time examined	Reticular Cells	Hemohistioblasts	Hemocytoblasts	Myeloblasts	Promyelocytes	Neutrophils: Myelocytes	Neutrophils: Metamyelocytes	Neutrophils: Stab	Neutrophils: Polymorphonuclear	Eosinophils: Myelocytes	Eosinophils: Metamyelocytes	Eosinophils: Stab	Eosinophils: Polymorphonuclear	Basophils	Prolymphocytes	Lymphocytes	Promonocytes	Monocytes	Megakaryoblasts	Megakaryocytes	Proerythroblasts	Basophilic erythroblasts	Polychromatophilic erythroblasts	Basophilic normoblasts	Polychromatophilic normoblasts	Orthochromic normoblasts	Plasma Cells: Erythrocytic series	Plasma Cells: Myelocytic series
Before experiment	-	-	2	2.4 4.4	0.8	4.8 (14.8)	9.2	21 (44.4)	23.4	1	1.4	2.6 (5.2)	0.2	0.2		1	-	0.4	-	0.2	3.4	2.8	9 (27.4)	0.4	6	5.8	0.4	0.4 (0.8)
After phenylhydrazine poisoning	-	-	7.4 (10.4)	3	2	0.2 (5.6)	3.4	10 (26)	16	0.4	0.6	0.8 (2.2)	0.4	-		-	-	-	-	1.2	7.6	8	12 (49.6)	2	18	2	1.8	3.2 5
During restoration of hematologic indices	0.2	1.2	3.2 (7.2)	2.6	2.8	5.2 (16)	8	20.6	22.8 (43.4)	1.2	0.8	0.6 (3)	0.4	-	0.8	1.4	0.2	0.4	0.2	0.4	0.2	2.8	6 (26.2)	3.2	8	6	0.4	0.4 (0.8)
After "electrocollision"	-	-	11.4 (16.4)	5	3.4	0.6 (12)	8	19.6	22 (41.6)	0.6	1.6	3.8 (6.4)	0.4	0.6	-	0.6	-	0.2	0.4	0.4	1.4	1.6	7 (20.8)	-	6	4.8	0.6	- (0.6)

in macrocytic anemia resulting from phenylhydrazine poisoning.

A similar pattern was established with regard to the peripheral blood composition. By the 30th-35th day after phenylhydrazine poisoning, a typical picture of hemotoxic anemia had developed. The hemoglobin content fell to 28-32%, and pronounced leukocytosis and reticulocytosis were observed. Collapse of higher nervous activity was induced after 35-44 days, when the hematologic indices were almost completely restored.

The results obtained showed that the "collision" acted as a stimulus which, to a certain extent, reproduced the blood picture typical of the previously existing hemotoxic anemia. This was more true in the case of the leukocyte and reticulocyte indices than in that of the erythrocyte and hemoglobin indices.

The question of the mechanism of hemotoxic anemia development has not yet been solved – it is uncertain whether hemolytic poison acts directly on the mature elements circulating in the blood or whether it acts through the nervous system.

Our investigations permit the hypothesis that trace reactions remain after the resolution of hemotoxic anemia, due to which hemopoietic changes observed during the anemia can be reproduced according to many hematologic indices by altering the functional activity of the cerebral cortex (in this case, by "electrocollision"). In the pathogenesis of certain anemia conditions, therefore, particular significance should be attached to disturbances of cortical regulation.

SUMMARY

As established by experiments, disruption of the higher nervous activity in dogs ("electrocollision") is accompanied by the change of the regeneration processes, especially of the white bone marrow series. Simultaneously there was a greater discharge of the white blood elements into peripheral blood. Functional disturbances of the higher portions of the CNS provoked by the "electrocollision" method against the background of phenylhydrazine poisoning led to some changes in the bone marrow hemopoiesis and in the peripheral blood composition, in many

ways similar to those occurring in the blood system during development of macrocytic hemolytic anemia.

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CHANGES IN BODY TEMPERATURE AND OXYGEN CONSUMPTION IN RABBITS FOLLOWING THE ADMINISTRATION OF SODIUM AMYTAL

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(Presented by AMN SSSR Active Member S. V. Anichkov)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 52, No. 7, pp. 48-53, July, 1961
Original article submitted June 9, 1960

Many investigations have demonstrated that the administration of barbiturates to animals causes a drop in body temperature [2-5,8,11] and considerable decrease in the basal metabolic rate [5-10]. However, to what extent the fall of body temperature effected by these narcotics is due to primary change in the basal metabolism or to primary disturbance in the thermoregulator mechanisms as a whole is not yet clear: in the latter case, the temperature drop could cause a secondary decrease in the metabolic level.

To solve these questions, we performed experiments comparing the changes which took place in the body temperature and oxygen consumption of rabbits, making our determinations at the shortest possible time intervals after the injection of sodium amytal.

METHOD

The body temperature of the animals was taken in the rectum, and the oxygen intake over ten minute periods was determined in a Renier and Reise type closed system as modified by P. N. Veselkin [1]. Sodium amytal was injected subcutaneously in doses of 40-200 mg/kg of a 5% aqueous solution.

RESULTS

Only slight fluctuations (up to 0.5 deg in 21 cases) of the body temperature, taken every hour for five to seven hours, were observed in 15 rabbits (28 experiments), while a space of 0.8 deg between the highest and lowest body temperature was only observed in seven experiments during the observation period.

After the subcutaneous injection of 40 mg/kg sodium amytal (12 experiments), the rabbits became sluggish and lay on their sides for 2-6 hr, but reacted vigorously when touched and, if pushed, crawled away, dragging their hind legs. In eight experiments, the body temperature dropped 0.4-5.4 deg after two to three hours. In two cases, there was practically no drop in temperature, while in the other two experiments, the body temperature rose 0.5 deg.

In nine rabbits, the oxygen consumption was determined hourly immediately after the temperature was taken. The experiments showed that the 40 mg/kg dose of sodium amytal reduced oxygen consumption 19-38% from the original level, the greatest decrease being observed after 1-2 (sometimes 3-4) hr. Oxygen consumption returned to the original level after 2-6 hr. In one experiment, no reduction of oxygen consumption occurred. On the whole the body temperature decreased more slowly than did the oxygen consumption, its maximum decrease often coinciding with the start of a relative increase in the metabolic level. In several cases, however, full restoration of the metabolic level was not observed until after equalization of the temperature curve.

In the individual experiments, we could observe no direct relationship between the changes in body temperature and metabolic level. Sometimes the body temperature even increased after the sodium amytal injection, although the oxygen consumption decreased.

Although the change in body temperature was comparatively uniform as to time and degree, the dynamics of the metabolic decrease could vary sharply (Fig. 1).

On the whole, the experiments in which 50, 100 and 200 mg/kg doses of the narcotic were administered

produced the same results.

The subcutaneous injection of 100 mg/kg sodium amytal (five experiments) caused deep sleep lasting about 5-6 hr (rabbits reacted weakly or not at all to a needle prick, although the corneal reflexes were retained) after an initial, brief period of excitation. The body temperature fell 3.6-7.8 deg after 4-6 hr, while oxygen consumption decreased 43-58% after only 1-4 hr. In three experiments, the original level of oxygen consumption was restored after 5-6 hr, but restoration of the original body temperature took 7-9 hr.

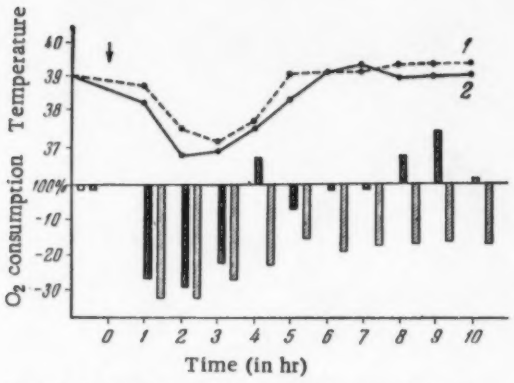

Fig. 1. Changes in body temperature and oxygen consumption in two rabbits after subcutaneous injection of sodium amytal in a dose of 40 mg/kg. Solid line and black columns give data for rabbit No. 1; dotted line and striped columns, for rabbit No. 2; arrow indicates sodium amytal injection.

No direct correlation between the changes in the general metabolic level and those in the body temperature was observed in these experiments either. Fig. 2 presents the results of two experiments by way of example.

Fig. 2 shows that the almost identical changes in oxygen consumption which occurred in the two rabbits following the administration of 100 mg/kg sodium amytal were attended by completely different changes in the temperature curves.

Six rabbits were given the narcotic in a dose of 200 mg/kg; three of these died within the first two hours after the injection, two rabbits died after two hours and one died after five hours. In these rabbits, the oxygen consumption (determined continuously except for the short intervals necessary for measuring the body temperature) fell considerably after about an hour and remained reduced until the animal's death. The temperature drop developed later in these experiments also. When the animals died, the level of oxygen consumption was still relatively high (43-50% of the original 10-15 min before death), and the body temperature was 33-36°.

After the administration of a uniform dose of sodium amytal, therefore, both the decrease in body temperature and the reduction of oxygen consumption varied greatly in different animals, evidently depending on the individual sensitivity of the animals to the barbiturate. When the dose of the narcotic was increased, however, both the hypothermia and the decrease in oxygen consumption were generally intensified – to a marked degree, according to the average data (Fig. 3).

The experiments also indicated that, in most of the experiments, the oxygen intake decreased before the body temperature, and that this precedence was retained during the restorative period. This gives reason to conclude that the fall of body temperature effected by sodium amytal to a certain extent results from a primary decrease in the metabolism. It is clear, however, that the degree to which thermogenesis diminished in most of our experiments could not itself cause such a sharp drop of body temperature if the heat losses were confined proportionately to this. The fact that no direct correlation was observed between the degree of decrease in body temperature and that in metabolism in any experiment also indicates that disturbance of the mechanism of thermoregulation must play an important part in the mechanism of the hypothermia development as well as the decrease in thermogenesis. But there are not sufficient grounds to consider the developing hypothermia the cause of the reduced metabolic rate either.

The data obtained brought up the question of just what, exactly, is the nature of barbiturates' effect on the thermoregulator centers. It was interesting in this connection to determine whether the ability to respond to the injection of pyrogenic agents by a rise of body temperature is retained by rabbits under the influence of barbiturates. In previous works [2,3], we demonstrated that the administration of a bacterial pyrogen to dogs during amytal hypothermia caused as sharp (and sometimes an even sharper) rise of body temperature as in the control experiments in which the dogs had not first received the narcotic. This reaction was particularly pronounced in dogs deprived of sight, smell and hearing; the reaction of such animals to pyrogens was also more acute under normal conditions. On the basis of these data, we concluded that the sensitivity of the thermoregulator center to a pyrogenic stimulus is higher in dogs under conditions of amytal sleep.

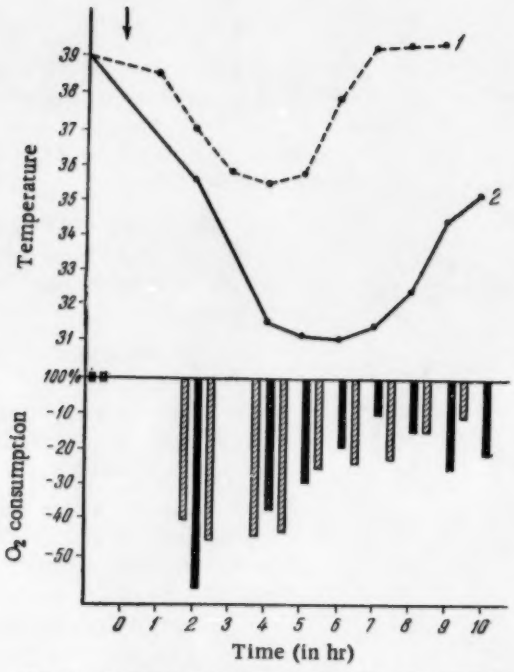

Fig. 2. Changes in body temperature and oxygen consumption in two rabbits after subcutaneous injection of 100 mg/kg sodium amytal. Symbols the same as in Fig. 1.

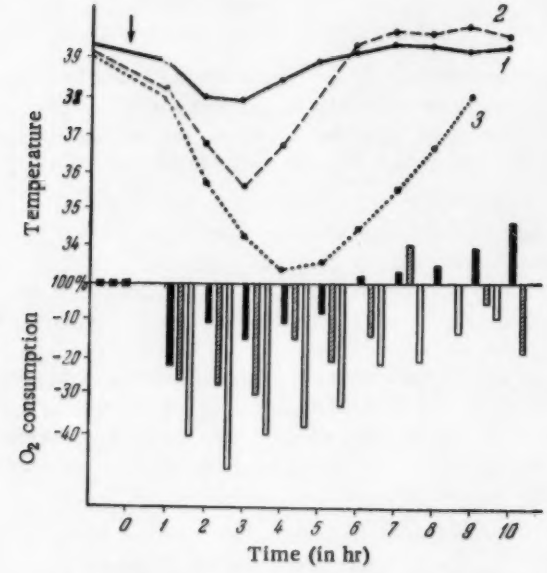

Fig. 3. Changes in body temperature and oxygen consumption after subcutaneous injection of sodium amytal in different doses (average data). Temperature curves: 1) 40 mg/kg; 2) 50-55 mg/kg; 3) 100 mg/kg; oxygen comsumption: black columns - 40 mg/kg; striped columns - 50-55 mg/kg; white columns - 100 mg/kg; ↓- sodium amytal injection.

To determine whether this conclusion held true for other types of animals also, we conducted 12 experiments in which a culture of B. mesentericus was injected (subcutaneously in a dose of 2 ml/kg) into rabbits which had received 40 mg/kg sodium amytal 2-3 min before the injection of the pyrogenic agent. After a period of variably pronounced, initial hypothermia, the body temperature increased considerably (an average of 1.1 deg) in all but one of these experiments, while in only 3 out of 12 control animals did the temperature rise much higher than the original level and then, much later in the experiment (see table).

Greatest Fall and Subsequent Rise of Body Temperature in Relation to Original Level in Rabbits after the Subcutaneous Injection of Sodium Amytal (40 mg/kg) and a Culture of B. mesentericus (2 ml/kg)

Change in body temperature after sodium amytal injection (control)			Change in body temperature after injection of sodium amytal and B. mesentericus culture		
Expt. No.	after 2-3 hr	after 5-10 hr	Expt. No	after 1-3 hr	after 5-9 hr
1	—	+0.5°[1]	13	—	+0.7° (5)[1]
2	−0.8° (3)	—	14	—	+1.1° (5)[1]
3	−5.4° (3)	—	15	−2.9° (2)	+0.9° (8)
4	−3.2° (3)	+0.2° (8)	16	−4.7° (3)	+0.1° (8)
5	−2.2° (2)	+0.4° (7)	17	−1.1° (3)	+1.5° (6)
6	−1.9° (3)	+0.3° (9)	18	−1.4° (2—3)	+1.3° (6)
7	−0.4° (2—3)	+0.3° (7)	19	−0.6° (2)	+0.7° (6)
8	−0.4° (2—3)	+0.2° (8)	20	−0.6° (2)	+1.4° (6)
9	−0.9° (3)	+0.1° (7)	21	−0.3° (1)	+2.0° (6)
10	−0.9° (2—3)	+0.6° (10)	22	−0.9° (2)	+1.5° (7)
11	−0.9° (2)	—	23	−0.5° (2)	+1.1° (5)
12	—	+0.5° (5)	24	−0.3° (1)	+1.2° (9)
Average	−1.4°	+0.3°		−1.1°	+1.1°

Note: Figures in parentheses show hour of maximum fall or rise of body temperature after the injections.

[1] Experiments discontinued after five hours.

Therefore, the sensitivity of thermoregulator centers to inadequate, "extraordinary" stimuli under conditions of amytal narcosis is also true in the case of rabbits. At the same time, the thermoregulator apparatus reacts much less strongly to the adequate stimuli developing in connection with the reduced thermogenesis and hypothermia, at least during the early stages of the barbiturate's effects. The fact that the temperature was restored in several experiments before the original metabolic level indicates that, at the later stages of the narcotic's effect, the thermoregulator apparatus begins to react to adequate stimuli as well, by equalizing the body temperature in spite of still reduced thermogenesis.

SUMMARY

In comparing the changes of body temperature and of oxygen intake in rabbits after sodium amytal injection no direct relationship was noted between the changes of the BMR level and body temperature (Fig. 1).

Sodium amytal given in a dose of 40 mg/kg reduces the oxygen intake by 19-38% with respect to the inital level. The highest reduction occurred in 1-2 (at times in 3-4) hours. With increase of the dose of this narcotic (100, 200 mg/kg) there are seen hypothermia and a drop of oxygen intake (Fig. 3).

Body temperature dropped in 4-6 hours by 3.6-7.8°C, whereas oxygen intake by 43-58% already in 1-4 hours.

Oxygen intake decreases in the majority of experiments earlier than body temperature. This leads to a conclusion that reduction of body temperature under the action of sodium amytal is to some extent the result of primary BMR reduction.

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THE ROLE OF ASCORBIC ACID IN THE MECHANISM OF ARTIFICIAL HYPOTHERMIA DEVELOPMENT

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 52, No. 7, pp. 54-57, July, 1961
Original article submitted July 26, 1960

Ascorbic acid plays an important part in the vital activity of the organism, and disturbance of its metabolism has serious consequences. Ascorbic acid actively participates in the formation of the adrenal cortical and medullary hormones [6,8,9,14,18,21], which are produced intensely when the organism is acted upon by extraordinarily strong stimuli. The brief application of a cold stimulus causes a marked decrease in the ascorbic acid content of the adrenal glands [2,10,12,13,16,17,20]. When the cold influence is prolonged, if the animals adapt to the low temperature conditions, the amount of ascorbic acid in the adrenal glands increases considerably [12,15]. When the animal organism is saturated with ascorbic acid, the usual hypertrophy of the adrenal glands in response to a cold stimulus is not observed [12], and the animals become more resistant to cold. The development of hibernation depends to a large extent on the ascorbic acid content of the blood and tissues; animals not sufficiently saturated with ascorbic acid (gophers) do not fall asleep [1].

All these data indicate the essential role of ascorbic acid in the metabolic processes with the action of cold stimuli. This work deals with the role of ascorbic acid in the mechanism of artificial hypothermia development.

METHOD

We used the following method [19] to quantitatively determine ascorbic acid: a trichloracetic extract of the blood or adrenal tissue was shaken with activated charcoal to oxidize the ascorbic acid into dehydroascorbic acid. If thiourea was present, phenylosazone formed when the charcoal filtrate was incubated with 2,4-dinitrophenylhydrazine. The addition of an 85% sulfuric acid solution caused a yellowish-red color to appear, the intensity of which was colorimetrically measured on a FÉK-M photoelectrocolorimeter. The ascorbic acid content of the blood and adrenal glands was calculated by comparison with a colorimetric curve constructed from a standard solution of ascorbic acid.

We examined 77 rabbits of both sexes, 13 of which served as the control. Artificial hypothermia was induced by the method employed in the clinic directed by V. I. Kazanskii [3,4,7]. The material was processed by the minor choice method of variational statistic investigation, determining the average indices, their average errors and the significance of the changes t. The changes were considered to be significant if $t \geq 3$.

RESULTS

Our analysis of the material showed that during the fall of body temperature, when the blood and adrenal glands of the animals were taken for examination, specific patterns of change could be established within certain temperature ranges. We divided the whole experimental temperature interval accordingly into three unequal parts: 31-26, 26-25 and 25-23 deg. We then proceeded to evaluate the changes which occurred in the ascorbic acid content of the adrenal glands and blood and in the weight of the adrenal glands during each of these temperature ranges.

The experimental results are shown as average indices in the table.

Under conditions of moderate hypothermia, i. e., within the 31-26 deg temperature range, we observed a

rather pronounced and statistically wholly significant decrease in the ascorbic acid content of the adrenal glands and a concurrent increase of its content in the blood, attended by a slight, statistically insignificant increase in the weight of the adrenal glands.

Change in the Ascorbic Acid Content of the Adrenal Glands and Blood and in the Weight of the Adrenal Glands in Rabbits During the Development of Artificial Hypothermia (in Average Indices)

Number of experiments	Range of rectal temperature	Ascorbic acid in adrenal glands (in mg%)	Ascorbic acid in blood (in mg%)		Weight of adrenal glands (in mg)
			Norm	Hypothermia	
13	37—38° (control)	260.0±38.0	—	—	110.07±8.43
41	31—26°	133.3±9.2 (t =3.24)	0.80±0.07	1.65±0.07 (t =8.5)	134.43±13.3 (t =1.55)
13	26—25°	85.07±14.3 (t =4.31)	0.66±0.1	1.85±0.1 (t =8.5)	68.6±11.5 (t =2.90)
10	25—23°	246.0±19.9 (t =0.33)	1.08±0.1	1.32±0.13 (t =1.5)	112.8±7.8 (t =0.24)

When hypothermia increased to the 26-25 deg range of temperatures, a further and more definite decrease in the ascorbic acid content of the adrenal glands was observed, attended by a sharp increase in its content in the blood. These changes from the original levels were found to be fully significant. In this temperature range, however, no increase in the weight of the adrenal glands attended their loss of ascorbic acid; on the contrary, their average weight was much less than it had been in the temperature range of 31-26 deg and substantially lower than the average weight of the adrenal glands in the control experiments.

In the temperature range of 25-23 deg, under conditions of deep hypothermia, the rather substantial changes observed in the experimental indices were the reverse of those previously noted: the average ascorbic acid content of the adrenal glands increased considerably, while the saturation of the blood with ascorbic acid decreased sharply, approximating the average indices determined at normal body temperature. The average weight of the adrenal glands increased until it approximated that of the control animals.

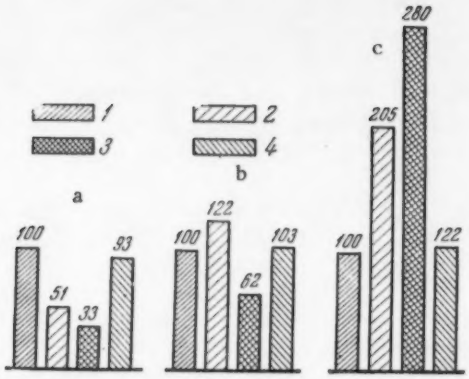
The figure shows the changes in the ascorbic acid content of the adrenal glands and blood and in the weight of the adrenal glands in the experimental temperature ranges, expressed in percent of the original levels or the control experiments.

Under our experimental conditions, premedication with a lytic cocktail considerably inhibited, but did not completely depress the organism's reaction to the cold stimulus.

In the 31-26 deg range of temperatures, there was a moderate increase in the weight of the adrenal glands. The adrenal function exhibited the adaptation syndrome, and the ascorbic acid in the adrenal glands was utilized in the production of steroid hormones. The increase in the ascorbic acid content of the blood could indicate that the amount of this substance required by the organs and tissues had decreased considerably due to the reduced intensity of the metabolism and the diminished expenditure of energy [5].

When hypothermia passed into a temperature range of 26-25 deg, premedication with a lytic cocktail could no longer maintain the hypophysial-adrenal system in a state of moderate stress. The ascorbic acid content of the adrenal glands decreased sharply; ascorbic acid was not, however, utilized by the adrenal glands to synthesize steroid hormones due to the considerable disturbance of the adrenal function. This is indicated by the acute decrease in the weight of the adrenal glands. Excessive amounts of ascorbic acid passed from the adrenal glands into the blood.

The further development of hypothermia to a 25-23 deg range of temperatures, according to the literature



Dynamics of change in ascorbic acid content under conditions of hypothermia: a) ascorbic acid content of adrenal glands (in mg%); b) average weight of adrenal glands (in mg); c) ascorbic acid content of blood (in mg%); 1) norm; 2) 31-26 deg; 3) 26-25 deg; 4) 25-23 deg.

data [11, 22], results in depression of the function of the higher, regulatory sections of the hypophysial-adrenal system, and this, evidently, to a large extent adapts the adrenal function to conditions of deep hypothermia. This is shown by the increased ability of adrenal gland tissue to concentrate ascorbic acid and by the partial restoration of their synthetic ability, which leads to an increase in their weight. It is possible that the ascorbic acid in the blood decomposes under these conditions, changing into the oxidized, dehydroascorbic form [2].

At this stage of hypothermia, the apparent equilibrium of the adrenal function is by no means stable, but rather indicates areactivity of the organism and the acute depression of all the vital functions; this leads to disturbance of the activity of the respiratory and cardiovascular systems, which is a well-known and frequent cause of death in patients or experimental animals operated upon under deep hypothermia.

All these data further confirm the essential role of ascorbic acid in the development mechanism of hypothermic conditions.

SUMMARY

The change of ascorbic acid content was studied in adrenal glands and blood of rabbits during the development of artificial hypothermia; changes occurring in the weight of adrenal glands were recorded as well. A reduction of ascorbic acid content in the adrenal glands takes place in conditions of moderate hypothermia at temperatures ranging from 31 to 26°C; there was also a moderate increase in the weight of the glands and a rise of ascorbic acid content in the blood. With intensification of hypothermia at temperatures ranging from 26 to 25°C a marked drop of ascorbic acid content in adrenal glands is accompanied by a reduction of their weight and discharge of ascorbic acid into the blood. In deep hypothermia (25-23°C) the amount of ascorbic acid in adrenal glands rises, their weight increases, while the blood ascorbic acid content declines.

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EFFECTS OF SOME NUCLEIC ACID COMPONENTS AND OF THEIR PRECURSORS ON INCORPORATION OF [1-C^{14}] GLYCINE AND [1-C^{14}] TYROSINE INTO THE PROTEINS OF NORMAL AND TUMOR TISSUE

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 52, No. 7,
pp. 58-61, July, 1961
Original article submitted July 22, 1960

The researches of the past few years have contributed valuable data to our knowledge of the role of nucleic acid in protein synthesis, and some of the mechanisms involved have been investigated. Although the first researches establishing a connection between the nucleic acid content of cells and the intensity of protein synthesis were begun less than 20 years ago, a number of postulates then advanced, on the basis of certain experimental findings, have since undergone revision. Thus, for example, the original view that deoxyribonucleic acid (DNA) is the directive agent in the synthesis of proteins has now been abandoned by the majority of workers, who consider that protein biosynthesis in the cytoplasm is connected directly with ribonucleic acid (RNA), and only indirectly with DNA.

Two recently published review articles [2,3] have been devoted to interrelations between protein synthesis and nucleic acids. Although many aspects of the role of nucleic acids in protein synthesis still await clarification, there can be no doubt that they are interrelated, and that the process proceeds at both the high-and the low-molecular level.

We were interested in investigating the part played by certain purine and pyrimidine bases of nucleic acids, and of their precursors, in the synthesis of protein. In the present research we examined the effects of aspartic acid, ureidosuccinic acid, and uracil (each of these substances being the precursor of the succeeding one) on incorporation of [1-C^{14}] glycine and [1-C^{14}] tyrosine into the proteins of slices of normal tissues, transplanted rat tumors, and ascitic tumor cells of rats. We also examined the effects of mixtures of pyrimidines (uracil, cytosine, thymine), and of each of these pyrimidines separately.*

METHOD

We examined the livers of normal rats and of rats with transplanted tumors, viz., rat sarcoma 45, and rat ascitic hepatoma (Sagedale strain). The experimental systems had a total volume of 5 ml, and contained 0.5 g of liver slices, 4 ml of Krebs-Ringer phosphate solution, 0.2 ml of various adjuvants, and 0.4 ml of [1-C^{14}] glycine or [1-C^{14}] tyrosine solutions, of an activity of 100 000-300 000 c.p.m. The systems were incubated in Warburg vessels for an hour at 38°. The slices were then washed with the same saline solution until the washings showed absence of radioactivity, when they were ground up with 2.5 ml of 0.2 N $HClO_4$. The control systems either did not contain additives, or these were introduced together with the $HClO_4$ before incubation. The homogenates were centrifuged, and the deposits were again treated with 0.2 N $HClO_4$. The supernatant solutions were made neutral with KOH, and the radioactivity of the acid-soluble extractives was measured. Nucleic acids were extracted from the sediments with 0.2 N $HClO_4$ at 90° for 30 min. Lipids were extracted by heating for 3 min with a 3:1 alcohol-ether mixture. The residues were washed with alcohol and ether, and dried.

Radioactivity was measured by means of a MST-17 end-window counter, in a Type B apparatus. The substances under examination were added to the systems at a concentration of $2 \cdot 10^{-3}$ M. Ureidosuccinic acid was synthesized according to Nyc and Mitchell [4], and its purity was checked by melting point, analytical, and chromatographic data [1].

* M.B. Sapozhnikova participated in the research.

RESULTS

The data of Table 1 illustrate the effects of ureidosuccinic acid on incorporation of [1-C^{14}] glycine into sarcoma 45 slices and into liver slices from tumor-bearing rats.

TABLE 1. Effect of Ureidosuccinic Acid on Incorporation of [1-C^{14}] Glycine into Proteins, Nucleic Acids, and Acid-Soluble Compounds of Slices of Liver and Rat Sarcoma 45 (counts per minute per 10 mg of protein)

Tissue	Protein		Nucleic acids		Acid-soluble compounds	
	Control	Experimental	Control	Experimental	Control	Experimental
Liver	130	52	37	32	384	291
	144	78	33	28	410	294
	249	181	55	47	549	405
	158	90	28	22	440	260
	215	167	66	64	866	671
Mean percentage inhibition	36.6 (60—22)		13.6 (21.4—3.2)		27.6 (41—22.6)	
Sarcoma 45	960	750	186	160	949	1 524
	505	369	99	90	990	1 084
	1 034	880	184	198	985	1 315
	988	630	159	160	1 015	1 621
	839	568	160	150	—	—
Mean percentage inhibition	26.4 (36.3—14.9)		5.8		+29	

Note: The figures in parentheses indicate the range of variation of percentage inhibition (the values for the control systems, not containing ureidosuccinic acid, are taken as 100).

The results show that ureidosuccinic acid inhibited incorporation of [1-C^{14}] glycine into the proteins of sarcoma 45 slices, and of the liver of tumor-bearing rats, but had practically no effect on incorporation of glycine into the nucleic acids of sarcoma 45 tissue, and only a slight effect on incorporation into liver nucleic acids. The opposite results were found for the acid-soluble fractions of the tissues under examination. Whereas incorporation of [1-C^{14}] glycine into this fraction of the liver of tumor-bearing rats was inhibited by 27.6%, it was activated to the same extent in sarcoma 45 tissue. This activation may possibly be ascribable to the relatively greater utilization of ureidosuccinic acid for the synthesis of acid—soluble nucleotides in tumor tissue than in liver.

In our further experiments we used [1-C^{14}] tyrosine, with the object of getting a clearer picture of the effects on protein metabolism; tyrosine differs from glycine in not being utilized for the synthesis of nucleic acid components.

In the experiments on an earlier precursor of nucleic acid components we took aspartic acid, and, for an assessment of the specificity of its action, we compared the effects with those given by equimolar concentrations of glutamic, malic, and succinic acids (Table 2).

The data of Table 2 show that aspartic acid caused enhanced incorporation of [1-C^{14}] tyrosine into the proteins of sarcoma 45 tissue, in all the experiments, whereas (except for one experiment) the remaining acids gave practically no activation of tyrosine uptake, or even depressed it.

In our next experiments we examined the effect on incorporation of [1-C^{14}] tyrosine into protein of addition of nucleic acid pyrimidine bases, separately or together; for this purpose we used uracil, thymine, and cytosine, and their mixtures in equimolar proportions (Table 3).

The data of Table 3 show that of the pyrimidine bases examined, only uracil had any activating effect on incorporation of [1-C^{14}] tyrosine. The mixture of pyrimidine bases did not show any additive action.

TABLE 2. Effects of Various Acids on Incorporation of [1-C^{14}] Tyrosine into the Proteins of Slices of Rat Sarcoma 45 Tissue (c.p.m. per 10 mg protein)

Control (without additives)	Acids			
	aspartic	glutamic	malic	succinic
780	1 229	1 106	944	1 125
865	1 144	723	683	587
250	328	228	232	200
135	228	150	163	164
70	105	79	79	77

TABLE 3. Effects of Various Nitrogenous Bases of Nucleic Acids, and of Their Mixtures, on Incorporation of [1-C^{14}] Tyrosine into Proteins of Rat Sarcoma 45 and Rat Ascitic Hepatoma (c.p.m. per 10 mg protein)

Number of experiment	Control (without additives)	Uracil	Thymine	Cytosine	Mixture
1	67	98	60	70	77
2	84	102	70	90	72
3*	573	680	583	577	680

* Rat ascitic hepatoma cells were used in this experiment.

TABLE 4. Effects of Aspartic Acid, Ureidosuccinic Acid, and Uracil on Incorporation of [1-C^{14}] Tyrosine into the Proteins of Sarcoma 45 Slices (c.p.m. per 10 mg protein)

Number of experiment	Control (without additives)	Acids		Uracil
		Aspartic	Ureidosuccinic	
1	105	224	72	205
2	80	170	101	246
3	85	200	74	232
4	70	90	48	213

TABLE 5. Effect of Uracil on Incorporation of [1-C^{14}] Tyrosine into the Proteins of Liver Slices from Normal Rats (c.p.m. per 10 mg protein)

No. of experiment	Control	Experiment
1	110	93
2	61	78
3	81	90
4	81	87

In our next series of experiments we compared the effects of adding two pyrimidine precursors (aspartic and ureidosuccinic acids), and of adding uracil, on incorporation of [1-C^{14}] tyrosine into the proteins of sarcoma 45 slices (Table 4).

The data of Table 4 indicate that aspartic acid activated incorporation of tyrosine into proteins to a greater extent than in the experiments of Table 2; the activating effect of uracil was even greater, incorporation being as much as three times that of the control systems, in some of the experiments. The effect of ureidosuccinic acid on incorporation of [1-C^{14}] tyrosine into proteins was much the same as for [1-C^{14}] glycine (see Table 1), viz., incorporation of tyrosine was considerably inhibited.

We examined the effect of uracil on incorporation of [1-C^{14}] tyrosine into the proteins of liver slices from control rats (Table 5), for purposes of comparison with its effect on tumor tissue.

The data of Table 5 show that uracil has practically no effect on the incorporation of [1-C^{14}] tyrosine into the proteins of normal liver.

Ogata et al. [5] found that some of the pyrimidine bases of nucleic acids promote incorporation of [1-C^{14}] glycine into ribo- and deoxyribonucleoproteins of regenerating rabbit liver; this effect was much less pronounced in normal liver. Considerable stimulation of incorporation was given by orotic acid, which is a pyrimidine precursor. These authors attributed the effect to utilization of pyrimidines by proliferating tissue as precursors of nucleic acids.

We also found that in transplanted rat tumors uracil activates protein biosynthesis, as shown by incorporation of [1-C^{14}] tyrosine, whereas this activating effect was not found with normal rat liver. These findings are in agreement with published reports of the slight utilization of pyrimidines by normal tissues, as contrasted with their utilization by tumor tissue. Rutman et al. [6] found that active incorporation of labeled uracil took place in tumor tissues, but not in normal tissues. Of the compounds examined by us, uracil was the most active in stimulating protein synthesis. The effect of aspartic acid closely resembled that of uracil, both qualitatively and quantitatively; aspartic acid is one of the direct precursors of the pyrimidine bases of nucleic acids. Activation of protein biosynthesis in tumor tissue by some of the precursors of nucleic acids should, perhaps, be regarded as a consequence of stimulation of

nucleic acid production in such tissues, this, in turn, stimulating protein synthesis, rather than as a direct result of the action of the precursors.

An unexpected result was the absence of any stimulating effect of ureidosuccinic acid (a precursor of orotic acid) on incorporation of [1-C^{14}] glycine into proteins of tumor tissue; this finding may be due partly to utilization of glycine for the synthesis of nucleic acids.

SUMMARY

The effect on incorporation of [1-C^{14}]-glycine and -tyrosine into rat liver and rat sarcoma 45 proteins of nucleic acid pyrimidine bases, and of their precursors, aspartic and ureidosuccinic acids, has been investigated. Of the substances examined, only aspartic acid and uracil were found to activate incorporation of labeled amino acids into proteins.

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CHOLESTEROL METABOLISM, ACETYLATION PROCESSES, AND THIAMINE

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 52, No. 7,
pp. 62-64, July, 1961
Original article submitted July 15, 1960

Practically the whole of the carbon skeleton of cholesterol is made up of acetyl residues, which are condensed initially to give the aliphatic hydrocarbon squalene. This is then converted, by an oxidocyclization reaction, into the phenanthrene ring system of cholesterol [12]. The synthesis of cholesterol proceeds in practically all of the tissues of homeotherms, and the first stage of this process is closely bound up with generation of active acetyl groups, produced by other metabolic processes. In the oxidative degradation of carbohydrates, an intermediate product in the formation of acetyl coenzyme A is a compound of a two-carbon fragment with thiamine pyrophosphate [15]. There are grounds for believing that this intermediate may function as an acetylating agent, transferring ready-made acetyl to coenzyme A to the pyruvate oxidase system [11, 14]. Coenzyme A would here function merely in the secondary role of carrier. This formulation would to some extent explain why incorporation of C^{14}-acetate into cholesterol is unaffected by pantothenate deficiency [13]. The existence of a dependence of the process of acetylation of sulfanilamide on the state of thiamine metabolism has been reported [2]. We have shown, on the other hand, that diversion of acetyl residues for acetylation of isoniazid has an appreciable effect on cholesterol synthesis [5].

Published data on the effect of thiamine on cholesterol metabolism are both scanty and contradictory. I. A. Myasnikova [4] found that a single injection of thiamine into patients caused a fall in the level of blood cholesterol, whereas prolonged administration had the opposite effect. Thiamine retards the development of experimental hypercholesterolemia in rabbits [3]. The rate of synthesis of cholesterol in avitaminotic rats was doubled by thiamine injections [16]. On the other hand, the cholesterol content of the blood and tissues of B_1-deficient pigeons was found to be raised [1].

In this paper we present new data on the dependence of cholesterol biosynthesis in humans and experimental animals on the rate of simultaneously proceeding acetylation processes; the effect of thiamine on the connection between these processes is also examined.

METHODS AND RESULTS

Since isoniazid could affect cholesterol metabolism not only by diversion of acetyl residues, but also by interference with thiamine metabolism [6], we used a different drug, p-aminosalicylic acid (PAS), in our experiments. The use of this drug gave the following advantages: it is much less toxic than is isoniazid, for which reason it is used therapeutically in much higher dosage (12-15 g per diem), and 60-80% of the dose is eliminated in the urine as its acetyl derivative. Hence this drug provides a much more effective means of diverting acetyl groups than does isoniazid, the daily dosage of which does not exceed 1.5-2 g for humans. Apart from this, PAS has a much smaller effect on thiamine metabolism [7].

Working together with M. M. Korotkina, we found that the mean blood cholesterol content of patients suffering from pulmonary tuberculosis fell, after three days of administration of PAS at a rate of 12 g per diem, from 290 to 250 mg-%. That of experimental animals (20 rabbits) fell similarly from 95 to 78 mg-% after three days of administration of PAS (0.15 g per kg body weight). The fall in the blood cholesterol of rabbits was significant in 19 out of 20 animals (there was no change in one rabbit).

We found that when 20-40% glucose was injected together with thiamine (5-50 mg) into patients receiving PAS, the effect of the latter in lowering blood cholesterol was abolished, or, in some cases, even reversed. We performed a special series of experiments on animals, with the object of elucidating the relation between thiamine

TABLE 1. Blood Cholesterol of Rabbits (mg-%)

Rabbits given glucose		Rabbits given thiamine	
Before administration of PAS	After administration of PAS	Before administration of PAS	After administration of PAS
90	78	66	79
88	62	94	100
100	92	102	114
77	60	78	90
76	70	88	90
128	102	110	110
95	90	94	100
65	60	72	69
80	64	68	70
110	100	70	74
Mean 91	78	84	90

TABLE 2. Serum Cholesterol (determined by the method of V. A. Éngel'gardt and L. I. Smirnova) of Patients Suffering from Pulmonary Tuberculosis, Treated with Isoniazid

Name of Patient	Cholesterol content (mg-%)			
	Before treatment	On the 3rd day of treatment	On the 4th day of treatment, with thiamine injection	On the 6th day of treatment, without thiamine
K-i	161	130	148	140
S-o	168	126	144	130
B-o	139	96	130	120
K-sh	120	99	115	110
S-ch	134	119	150*	130
M-v	160	148	157*	136
Sh-a	134	111	136*	120
Ya-k	133	124	142*	121

* Cocarboxylase injections.

and cholesterol metabolism.

PAS was administered to two groups of ten rabbits each for 3 days, at a dosage rate of 0.2 g per kg body weight per diem. An intramuscular injection of 10 ml of 5% glucose was given at the same time to one group of rabbits, while the other group received a solution of 2 mg of thiamine chloride in 10 ml of physiological saline. Blood cholesterol was determined before, and after three days of administration of PAS (Table 1).

Glucose administered without thiamine did not affect the action of PAS on cholesterol metabolism: blood cholesterol fell in all cases. No such effect was observed when thiamine was given together with glucose, and in some cases there was even a rise in the blood cholesterol level. There can be no question of any antagonistic action between PAS and thiamine, since we had earlier observed a similar effect in patients receiving therapeutic doses of isoniazid. Because of the small number of such observations, we did not publish them earlier, without further experimental verification. The structures and mechanisms of action of PAS and isoniazid are quite different from each other. The only feature which they possess in common, in relation to cholesterol metabolism, is that they are both actively acetylated in the organism. Apart from this, isoniazid may inhibit phosphorylation of thiamine [6]. The data of Table 2 illustrate our observations on the serum cholesterol content of tuberculous patients on a daily dosage of 1.5 g isoniazid. On the 4th day they were given intramuscular injections of thiamine chloride (0.5 mg per kg body weight) or cocarboxylase (0.7 mg per kg body weight; the preparation was a mixture of thiamine phosphates,

containing 75% thiamine pyrophosphate).

The hypocholesterolemic action of isoniazid was abolished in all the eight patients examined by a single injection of thiamine, and even more markedly by thiamine phosphates. This effect of thiamine was no longer apparent on the day following its injection.

Thus these observations, made at different times, and with different drugs, which had the common property of being readily acetylated in the organism, made it evident that the mechanism of their action on cholesterol metabolism was basically the same in both cases. In this respect, the data derived from patients and from animal experiments were in complete consonance. Both PAS and isoniazid caused a definite drop in the level of blood cholesterol. This effect was not abolished by glucose, and, in individual cases, administration of thiamine during treatment with the chemotherapeutic preparations even raised the blood cholesterol level above the initial value.

We have found [8,9,10] that the free serum thiamine levels of patients suffering from hypertension and atherosclerosis were very high, as were also their transketolase and thiamine dehydrogenase activities. All this affords evidence of a general activation of thiamine metabolism, which is geared to a higher level of activity. We do not believe that this association between activation of thiamine metabolism and pronounced hypercholesterolemia encountered in the majority of individuals suffering from hypertensive disease can be purely fortuitous. It seems that thiamine is actively concerned in the generation of active acetyl, and that it promotes a preponderance of anabolic over catabolic processes of lipid metabolism. Not enough is as yet known about other pathways of lipid metabolism involving thiamine, and additional research on this question is required. In view of the results here presented we think it may be of practical importance to reconsider the question of the use of thiamine in the treatment of hypertensive disease.

SUMMARY

The effects of PAS and isoniazid on blood cholesterol are ascribed to diversion of active acetyl residues from cholesterol synthesis to acetylation reactions. Thiamine is thought to function as an activator of processes of lipid anabolism. The need for caution in the use of thiamine in treatment of hypertensive disease and atherosclerosis is indicated.

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THE GLYCOGEN CONTENT IN THE LIVER AND MUSCLES OF RATS IN RELATION TO THE TIME ELAPSED AFTER ADMINISTRATION OF CHLOROPROPAMIDE AND TO THE DURATION OF FASTING

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 52, No. 7, pp. 65-68, July, 1961
Original article submitted March 30, 1960

The antidiabetic sulfonamides decrease the liberation of glucose from the liver into the blood. This frequently leads to an increase in the glycogen content of the liver [1]. In conjunction to considerable increase in the glycogen content of the liver in some cases only slight enlargement of the liver [12] can be observed and sometimes no changes of that organ are found at all [9,10,11,14]. No changes in the glycogen content of the liver were found after 5-14 days of administration of one of the strongest antidiabetic preparations: metahexamide [8]. And what is more: under certain circumstances carbutamide even causes a decrease in the glycogen content of the liver [13]. The results of investigations concerning the glycogen content in the skeletal muscles are also of contradictory character. Administration of carbutamide either failed to change the glycogen content of muscles in dogs or even caused a slight decrease [13].

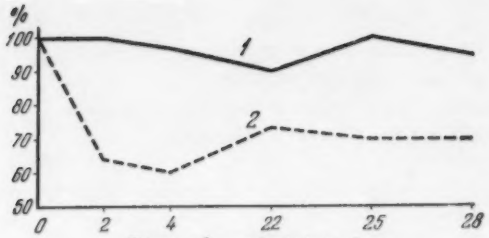
The contradictory data in the literature induced us to study the causes responsible for the different influence exerted by antidiabetic sulfonamides upon the glycogen content of the liver and the muscles.

In previous investigations [2-5] we had shown that butamide, cyclamide and chlorocyclamide exerted, when injected into rats for 5, 10 or 20 days, no influence upon the glycogen content of the liver within 15-17 hours after consumption of food and administration of sulfonamides. After five days of administration of cyclamide the glycogen content of the skeletal muscles decreased whereas administration lasting 10 or 20 days caused no changes in the glycogen content. Butamide and chlorocyclamide increased the glycogen content of the skeletal muscles whatever the time of administration. Butamide, chlorocyclamide and chloropropamide caused a marked increase in the glycogen content of the liver four hours after consumption of food and administration of sulfonamides without exerting any influence upon the glycogen content of the muscles.

In the present paper we studied the influence of chloropropamide (synthetized in the Ukrainian Institute of Experimental Endocrinology by T. F. Sysoeva and N. I. Makhnenko) upon the glycogen content of the liver and muscles, following up the pattern of changes over various periods, administering the drugs for one day or for many days and in relation to the time elapsed since the last consumption of food and the last intake of the antidiabetic substance.

METHOD

Male rats weighing 150-200 g served as control and experimental animals. They were given the standard diet consisting of roots (carrots and beets - 3 g), concentrated foods (oats, barley, bran, maize, millet, and sunflower seed-15 g), bread (15 g) and soup (50 g); the latter included meat (5 g), milk (8 g), semolina (5 g), salt and water. At 9-10 a.m. the animals were given the grain and the roots and at 1-2 p.m. the other ingredients of their ration. Chloropropamide was administered perorally in a dose of 100 mg per 1 kg body weight (in milk; with addition of a small quantity of gum arabic). Within a certain time after the last consumption of food and administration of chloropropamide the animals were decapitated; pieces of the liver and the m. gastrocnemius were excised to estimate their glycogen content by the method of Pflüger in a modification developed by us [6]. The blood sugar level was estimated by the method of Hagedorn-Jensen. The control animals were different from the experimental animals in the respect that they were not given chloropropamide. After each period of investigation groups of 5-10 experimental rats and 5-15 control rats were killed. The findings were evaluated statistically by the method of Fisher and



Changes in the blood sugar level under the influence of a single dose of chloropropamide against a background of 22-50 hours fasting (in per cent of the original value defined as 100). 1) Control animals, 2) experimental animals.

Student. The statistical significance of the difference P was calculated according to the table of Fisher [7].

RESULTS

The influence of a single administration of chloropropamide against a background of 22-50 hours' starvation.

Rats which had been fasting for 22 hours were given a single dose of chloropropamide and 2,4,22,25 and 28 hours later the blood sugar level and the content of glycogen in the liver and the skeletal muscles were estimated. Throughout the period in question the animals were not given any food and were kept in special cages provided with a double bottom securing the elimination of faeces.

The curve (figure) shows that the blood sugar level was in the control animals more or less stable whereas in the experimental animals the blood sugar level decreased considerably two and four hours after administration of chloropropamide respectively. Although subsequently the blood sugar level showed a slight increase it was still even after 28 hours far from reaching the original level.

The glycogen content of the liver showed a marked increase two and four hours after administration of chloropropamide respectively (Table 1). The difference between the findings obtained in the control and the experimental rats respectively proved to be statistically significant even after 22 hours, as P was < 0,02. At later periods of the experiments the glycogen content in the liver of the experimental animals was the same as that in the control animals. The glycogen content in the skeletal muscles remained unchanged at all periods of investigation.

TABLE 1. The Influence of a Single Administration of Chloropropamide Against a Background of 22-50 Hr Fasting upon the Glycogen Content in the Liver

Duration of fasting (in hr)	Time since chloropropamide administration (in hr)	Glycogen content of liver ($\bar{x} \pm S\bar{x}$)				
		Control animals	n	Experimental animals	n	P
22	—	223±9.7	6	—	—	—
24	2	214±5	6	305±20	6	<0.001
26	4	200±10.9	15	280±11.9	10	<0.001
44	22	200±9.6	12	242±10.6	6	<0.02
47	25	118±15.6	12	92±20.2	6	0.3
50	28	100±16	12	97±16	6	—

Remark: $\bar{x} \pm S\bar{x}$ represents the mean arithmetical ± deviation of the means; n-the number of experiments; P-the criterion for the statistical significance of the difference.

Administration of chloropropamide for ten days and the content of glycogen in the tissues 4-45 hours after the last consumption of food and administration of the preparation.

The rats were given chloropropamide daily for ten days. On the last day the animals were kept in the fasting cage and were given the preparation. The blood sugar level and also the concentration of glycogen in the liver and the muscles of these animals were investigated 4,6,8,25 and 28 hours (first series of experiments) and also 17,19,21,42 and 45 hours (second series of experiments) after the last feeding and the last administration of chloropropamide. Within 4-28 hours the blood sugar level showed a marked decrease which averaged 33%; the fall was much less in rats of the second series of experiments after 17-19 hours.

The data set forth in Table 2 show that the glycogen content in the liver increased considerably 4,6 and 8 hours after the last consumption of food and the last administration of chloropropamide. At the other intervals the glycogen content was the same as in the control animals. The glycogen content in the skeletal muscles of the experimental animals was somewhat higher at all periods of investigation. This difference, however, was significant only 8,17 and 19 hours after the last consumption of food and last intake of chloropropamide. Later the glycogen content of the

TABLE 2. The Influence of Administration of Chloropropamide Continued for Ten Days upon the Glycogen Content in the Liver and Muscles 4-45 Hours after the Last Consumption of Food and Last Administration of the Preparation (Average Findings Obtained on Six Animals; the Data Referring to 45 Hours Represent the Average Findings Obtained on Five Animals)

Time since intake of food and chloropropamide (in hr)	Glycogen ($\bar{x} \pm S\bar{x}$)					
	Liver			Muscles		
	Control animals	Experimental animals	P	Control animals	Experimental animals	P
4	1085±35.5	2330±62.3	<0.001	453±32.5	510±20	<0.3
6	1046±43.6	1906±24.1	<0.001	483±24.3	527±14.4	<0.1
8	945±43.5	1390±126	<0.001	403±10	729±32.4	<0.001
17	475±24	490±11	<0.5	545±22.9	663±25.6	<0.01
19	465±36	487±19	—	540±20.7	600±30.7	<0.001
21	250±22.5	250±23.4	—	455±14.8	477±20.4	—
25	226±13	224±13.5	—	400±12	410±8.7	—
28	210±10	222±13	—	420±12.8	500±32	—
42	190±8.3	170±20	—	450±14	440±12.8	—
45	133±9.2	127±9	—	427±14.3	417±14.3	—

Remark: $\bar{x} \pm S\bar{x}$ represents the mean arithmetical ± deviation of the means; P represents the criterion for the statistical significance of the difference.

muscles approached that of the control animals.

The data quoted above show that the glycogen content in the liver and the muscles depends not only on the action of chloropropamide but also to a considerable degree on the time through which the experimental animals were fasting. After administration of chloropropamide for ten days the glycogen content of the liver increases; this increase is particularly marked in the first eight hours after the administration of the preparation whereas the glycogen content shows no changes in the period between 17 and 45 hours after the last consumption of food and the last administration of the preparation.

It thus appears that the increase in the glycogen content of the liver observed after administration of chloropropamide for several days is the more intensive the shorter the time of fasting and the shorter the time elapsed after the last administration of the preparation. The influence of chloropropamide upon the glycogen content of the liver is the weaker the longer the time elapsed after the last administration of the preparation and the last consumption of food.

If only a single dose of chloropropamide is administered the glycogen content of the liver increases also after fasting lasting 24 and 26 hours respectively and two and four hours after the administration of the preparation. It thus appears that given fasting of equal duration the glycogen content of the liver depends on the time elapsed after the last administration of chloropropamide.

Chloropropamide also exerted a varying influence upon the glycogen content of the muscles depending on the duration of fasting. After fasting lasting four hours the glycogen content of the muscles showed a slight rise at almost all periods of investigation (first series) although the difference was statistically significant only after eight hours; it proved nevertheless to be increased under the influence of chloropropamide also after fasting lasting 17 and 19 hours respectively (second series). If the fasting lasted longer, chloropropamide exerted no influence upon the glycogen content of the skeletal muscles.

If one compares the effect of chloropropamide upon the blood sugar level and the glycogen content in the liver and muscles the absence of any regularity deserves attention. Chloropropamide decreases the blood sugar level independently of the time of fasting. At the same time the glycogen content of the liver and muscles may be increased or may be unchanged. For example, after a single administration of chloropropamide into rats which had been fasting for 22 hours the glycogen content in the liver increased 2-4 hours after the last administration of the preparation and remained subsequently unchanged notwithstanding the considerable decrease in the blood sugar level.

The glycogen content in the skeletal muscles did not change throughout the period of investigation.

The discrepancy between the decrease of the blood sugar level under the influence of chloropropamide and the glycogen content of the liver and muscles became particularly manifest if the preparation was administered into rats which had been fasting for four hours. In these animals the glycogen content showed a marked increase in the first 4-8 hours of action of the sulfonamide but later, notwithstanding the continued decrease in the blood sugar level, the glycogen content in the liver showed a marked decrease just as in the control animals. In the muscles on the other hand the glycogen content increases within 8-19 hours after the administration of the preparation.

The results obtained by us explain the discrepancy found in the literature concerning the influence of antidiabetic sulfonamides upon the glycogen content in the liver and muscles.

SUMMARY

A study was made of the effect of chloropropamide(100 mg per kg) on the blood sugar level and the content of glycogen in the liver and skeletal muscles of rats in 4,6,8,17,19,21,25,28,42 and 45 hours after the food intake was stopped and the preparation was administered. Marked reduction of the blood sugar level was noted for a period of 28 hours, whereas the glycogen content in the liver increased considerably only in 4,6 and 8 hours; in the muscles it increased in 8,17 and 19 hours. The content of glycogen in the liver and muscles depends not only on the chloropropamide action but also on the duration of the previous starvation.

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THE EFFECT OF ULTRAHIGH-FREQUENCY CURRENTS ON BLOOD SERUM PROTEIN FRACTIONS

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 52, No. 7,
pp. 69-72, July, 1961
Original article submitted July 28, 1960*

Data in the literature[1,2,5 and many other references] testify to the distinct, responsive reaction of the leucocytes and erythrocytes of an animal organism to the influence of an ultrahigh-frequency (UHF) electrical field. However, the effect of a UHF field on blood protein composition has been examined only incidentally and by few authors [3,6].

We felt it would be timely to carry out an experimental investigation to compare the effect of high-frequency currents, UHF, diathermy and d'Arsonval currents on the protein composition of rabbit blood.

METHOD

The study was carried out on 56 healthy rabbits which weighed 2.54 ± 0.25 kg each, and which were maintained under identical conditions, on a regular feeding regimen, during the experiment. The source of the UHF electrical field was a portable apparatus, the UVCh-1P or UVCh-4, the generators of which worked on a fixed wave of 6 m, with a therapeutic circuit capacity of 50 w. An air gap of 1–1.5 cm between the surface of the electrodes and the body of the animal was maintained during exposure. The electrodes were placed bitemporally in the region of the head, and bilaterally in the middle part of the trunk. As the source of the diathermy current we used the universal device UDL-200, which has a generator capacity of 200 watts. A current strength of 0.03–0.05 amp was applied. The electrodes were positioned bilaterally on the middle part of the trunk. For local d'Arsonvalization a portable apparatus with mushroom-shaped condenser electrodes, which was moved freely from one position to another along the flat surfaces of the trunk in the course of the experiment, was employed. During the experiment the rabbits were always secured in one position in a special wooden frame, in which they were also held during the blood extraction. The sample blood, withdrawn directly into a dry test tube, was taken from the local veins of the ear surface. Before beginning the experiment the total protein and protein fractions were measured five to seven times in each rabbit to establish individual normal values. Daily exposures were carried out for a period of 10 days; the exposures lasted 5 and 15 min. We assayed the blood protein composition during the exposures on the first, second, third, fifth, seventh and tenth days, before the exposure, and immediately after it, and also on the 15th and 20th days, when the exposures had been discontinued. The total protein was assayed refractometrically and the blood serum protein fractions were measured by paper electrophoresis. Conditions of separation: barbiturate buffer, pH 8.6, ionic strength 0.05–0.1, voltage 230-250 v, current strength 3.5–5 ma, time of separation 14-16 hours. We subjected the data obtained to statistical treatment of a corollary series. We referred to N. V. Luchnik's table [4] to test the reliability of the results and also the presence of reliable differences in the comparison of the individual indices. $P = 0.05$ was taken as the level of significance.

RESULTS

In the first series of tests, which were performed on 40 rabbits, divided into four groups, we carried out two

* Work materials were reported at scientific conferences at the Bashkirian Medical Institute on December 28, 1956 and April 16, 1959.

modifications of the exposure to the UHF currents: in the first, the period of exposure was 15 minutes; in the second, 5 minutes. The test results indicated that a 0.3 ml controlled blood sample at 1-2 day intervals over a period of 15-20 days revealed no marked effect on the content of total protein and protein fractions (see table). From the data of the table it is seen that during the influence of the UHF electrical field on the rabbit organism, a clearly indicated dysproteinemia sets in, which is expressed in a lowering of the content of the albumin and an elevation of the globulin fractions. A regular lowering of the blood serum albumin fraction, from the very first day after the action of the UHF field (for which a bilateral arrangement of the electrodes and an exposure of 15 minutes were employed), began in the animals; a maximum lowering was reached on the seventh day of the test. In comparison with the mean initial value, the relative content of albumins was lowered by 15.79% on these days. As a rule, the content of the α_1 globulin fraction increased by 33.76%, and the γ globulin fraction, by 41.12%. Although the content of the β globulin fraction was somewhat higher than the upper limit of the normal, this increase was not statistically reliable. The A/Γ ratio for these days reached a value of 1.13, in comparison with the original value of 1.69. The total protein content was increased slightly. The absolute content of the protein fractions changed in a manner analogous to the change in the relative indices of the proteinogram. Beginning on the tenth day, normalization started in the correlation of the blood serum protein fractions, with the exception of the γ globulin fraction, the content of which, in comparison with the original level, remained elevated by 37.65%. On the 20th day, when exposure had been discontinued, the correlation of the protein fractions returned to the original norm.

It was of interest to investigate how the duration of the UHF field exposure influenced the proteinogram. In order to do this, we studied the effect of the UHF fields in two groups of rabbits during 5 minutes exposure with bilateral and bitemporal positioning of the electrodes. As seen from the data of the table, the UHF field during the 5 minute action had a biphasic effect on the proteinogram of the rabbit blood serum. In the first phase the relative amounts of albumin were lowered, after the very first exposure to the UHF field, by 5.12%; the content of γ globulins

Changes in the Protein Fractions of Rabbit Blood Serum Due to the Action of UHF Fields

Duration of exposure (in min)	Time after exposure (in days)	Statistical index	Total protein (in g%)	Albumins	Globulins				A/Γ ratio
					α_1	α_2	β	γ	
				in relative percentages					
Before experiment (average of 72 determinations)		M	6.56	62.73	7.82	6.86	9.87	12.72	1.69
		$\pm\sigma$	±0.59	± 3.66	±1.01	±1.19	±1.26	±2.10	±0.12
15	1	M	6.79	59.93	7.93	6.63	10.61	14.90	1.49
		P	>0.05	= 0.01	>0.05	>0.05	>0.05	=0.001	>0.05
	3	M	7.06	57.76	8.86	6.42	10.66	16.30	1.36
		P	=0.001	= 0.01	>0.05	>0.05	>0.05	=0.001	>0.05
	7	M	7.04	52.83	10.46	7.29	11.47	17.95	1.13
		P	=0.001	=0.001	=0.01	>0.05	>0.05	=0.001	=0.01
	10	M	6.93	56.77	8.92	6.61	10.19	17.51	1.29
		P	=0.01	= 0.01	=0.01	>0.05	>0.05	=0.001	=0.01
	20	M	6.58	62.81	8.03	6.98	9.86	12.32	1.68
		P	>0.05	> 0.05	>0.05	>0.05	>0.05	>0.05	>0.05
Before experiment (average of 42 determinations)		M	6.51	57.35	8.82	7.56	10.54	15.73	1.33
		$\pm\sigma$	±0.54	± 2.53	±1.17	±0.97	±1.60	±2.65	±0.12
5	1	M	6.46	54.53	9.03	7.86	10.95	17.63	1.19
		P	>0.05	= 0.01	>0.05	>0.05	>0.05	=0.01	>0.05
	3	M	7.10	57.26	10.28	7.57	9.37	15.52	1.33
		P	=0.01	> 0.05	=0.01	>0.05	>0.05	>0.05	>0.05
	10	M	6.78	50.12	10.20	9.32	12.54	17.82	1.00
		P	>0.05	=0.01	=0.01	=0.01	>0.05	=0.01	=0.01
	20	M	6.49	56.65	8.97	8.40	10.31	15.67	1.31
		P	>0.05	> 0.05	>0.05	>0.05	>0.05	>0.05	>0.05

Note. M is arithmetical mean; σ—standard deviation; P—probability, equal to 0.05.

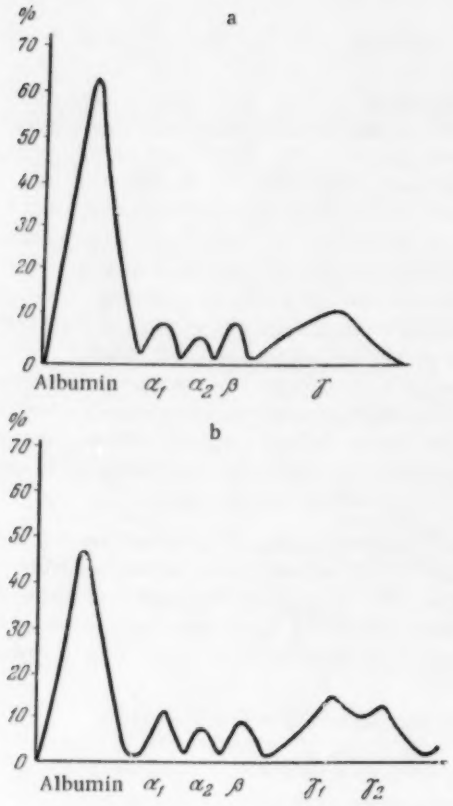

Fig. 1. Protein fractions of rabbit blood serum. a-Before the experiment, b- on the fifth day after daily exposure to the UHF electrical field.

rose by 12.07% in comparison with the initial value. On the third day, despite the extended action of the UHF field, the proteinogram returned to the initial norm and stayed at this level until the 10th day of the test. From the 10th day on, in the second phase, dysproteinemia once again set in, and was also expressed in a lowering of the albumins by 12.6% and an elevation of α_1 globulins by 15.64%, α_2 globulins by 23.28% and γ globulins by 13.28%. The A/Γ ratio was lowered to 1, in comparison with the original value of 1.33. On the 20th day the blood protein picture was once again the same as it was at the start.

Shifts in the fractional composition of the blood serum proteins brought about by the UHF electrical field, using a bitemporal placement of the electrodes, for both the 5-minute and 15-minute exposures, were analogous to the changes described above where a bilateral placement of the electrodes was used.

It should be noted that in all cases of exposure of the rabbits to the UHF field, we observed, after the very first session, the appearance on the electrophoretic curve, of one or, more often, two additional peaks, which were evidently the consequence of a separation in the γ globulin fraction (see figure). These complementary peaks in the electrophoregram were noted during the entire period of exposure , but no longer appeared on the electrophoregram obtained on the fifth day after cessation of the exposure.

A comparison of the rabbit proteinograms made at identical times after exposure to a UHF field, to a diathermy current, and to a d'Arsonval current revealed an especially great contrast in the biological effect of the UHF field. In the case of the UHF field, dysproteinemia appeared immediately after the first exposure, and a return to the initial value in the correlation of the protein fractions did not begin until the 20th day; however, the change in the proteinogram of the rabbits after exposure to the diathermy current and the d'Arsonval current was very slight and of short duration. The blood protein picture during exposure to the diathermy currents did not change until the 10th day; this was expressed in an increased content of albumins, and a lowering of the β and γ globulins. Normalization of the proteinogram took place on the 15th day.

During exposure to the d'Arsonval currents, dysproteinemia appeared within three days after the beginning of the action and was manifested in a lowering of the albumin content and an elevation of the γ globulins. On the fifth day, the correlation of the protein fractions returned to its original norm. Furthermore, despite the duration of the influence of the d'Arsonval currents, this correlation was within the limits of the initial value.

Thus, the data obtained indicate that the UHF electrical field is a powerful stimulus for the animal organism and evokes a definite change in the protein composition of rabbit blood serum. Dysproteinemia arising under the influence of a UHF electrical field is more profound and more lasting than the dysproteinemia after exposure to diathermy and d'Arsonval currents. In considering the significance of globulin in the immunity processes, it can be assumed that the beneficial effect of the UHF electrical field in therapy consists in the elevation of the globulin fractions, especially the γ globulins, and that this strengthens the defensive forces of the organism.

SUMMARY

The author studied the effect of ultra-high frequency field, diathermy and d'Arsonval currents on the blood serum proteins of rabbits by the method of paper electrophoresis. Dysproteinemia occurring in the action of an ultra-high frequency field is characterized by a reduction of albumin and a rise of globulin content, especially α_1

and γ globulins. This dysproteinemia is more marked and prolonged than that provoked by diathermy and d'Arsonval currents. Considering the great role played by the globulins in the immunity processes a conclusion may be drawn that the favorable effect of ultra-high frequency field in the treatment of various diseases consists of the rise of globulin fractions, especially of γ globulins, intensifying the protective body functions.

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HYPERKINESIS OF CENTRAL ORIGIN INDUCED BY TETRIDINE AND ITS INHIBITION BY ANTICHOLINESTERASE (CHOLINE-SENSITIZING) AGENTS

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 52, No. 7, pp. 73-75, July, 1961
Original article submitted July 2, 1960

Many researchers engaged in the study of effects induced by barbiturates have observed peculiar motor activity in both animals and people. This reaction is known as barbiturate, or stem, hyperkinesia and has been described in detail by I. V. Markova [4,6]; the same author has shown that stem hyperkinesia can be induced by other somnifacients (besides barbituric acid derivatives) and by anticonvulsants [5]. In experiments on white mice, I. V. Markova discovered that proserine [neostigmine]* (0.01-0.1 mg/kg) can be used to prevent and eliminate barbiturate hyperkinesis.

We discovered that tetridine (2,4-dioxy-3,3-diethyltetrahydropyridine), a pyridine derivative similar in structure and narcotic effect to barbiturates [1,7,8], induces stem hyperkinesia in different animals (frogs, mice, rats, rabbits and guinea pigs). We used medinal [barbital sodium], Nembutal and hexenal [hexobarbital] to demonstrate the similarity of barbiturate hyperkinesia to tetridine hyperkinesia.

The purpose of this work was to investigate the effect of anticholinesterase agents (proserine, eserine and phosphacol [diethyl p-nitrophenyl phosphate]) and cholinolytic agents (atropine, scopolamine, tropacine [ester of diphenylacetic acid hydrocloride], pentaphene [diethylaminoethyl ester of phenylcyclopentacarbonic acid], arpenal [acylated diethylpropylene diamine], methyldiphacyl [diphenylacetic acid β-diethylaminoisopropyl ester hydrochloride] and methyldiazyl [benzilic acid β-diethylaminopropyl ester hydrochloride]) on tetridine hyperkinesia in guinea pigs and white mice.

METHOD

All the compounds mentioned above were administered intraperitoneally to the guinea pigs and subcutaneously to the mice. Hyperkinesia was induced by tetridine, used in doses of 100-130 mg/kg for the guinea pigs and in doses of 150-200 mg/kg for the mice. The hyperkinesia induced by these doses lasted two hours or more.

Since hyperkinesia was particularly well defined in the guinea pigs, the majority of the experiments were performed on these animals; the anticholinesterase and cholinolytic agents were administered to them on a background of developed hyperkinesia, but to the mice before the administration of tetridine.

RESULTS

The administration of anticholinesterase agents (0.06-0.16 mg/kg proserine, 0.15-1.0 mg/kg eserine salicylate or 0.2 mg/kg phosphacol) to guinea pigs on a background of tetridine hyperkinesia completely inhibited the latter for 10-60 min (Fig. 1). This inhibition could be reproduced several times in the same animal (but was not attempted more than two to three times in our investigations because of the other effects induced by any additional injections–fasciculation, etc.).

The administration of cholinolytic agents (5 and 50 mg/kg atropine sulfate, 0.5 and 25 mg/kg scopolamine hydrobromide, 12.5 and 50 mg/kg tropacine, 10 and 15 mg/kg pentaphene, 40 mg/kg arpenal, 10 mg/kg methyldiphacyl or 10 and 20 mg/kg methyldiazyl) to mice 10-15 min before the tetridine injection did not prevent the development of hyperkinesia. This agrees with the data N. A. Kharauzov [9] obtained in his study of diphacyl's

*Here and hereafter the terms in brackets are the preferred or more commonly used English expressions for the immediately preceding Russian names [Publisher's note].

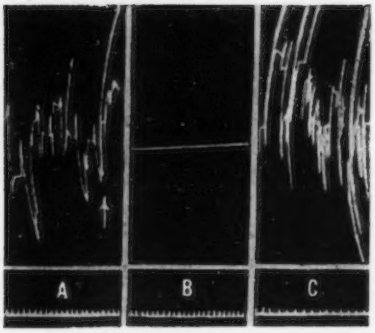

Fig. 1. Kymogram of hyperkinesia induced in a guinea pig by the intraperitoneal injection of 130 mg/kg tetridine. The animal is lying on its side, the recording apparatus connected to its hind leg. A) Before administration of eserine salicylate; B) 15 min after administration of 1 mg/kg eserine—total inhibition of hyperkinesia; C) 24 min after eserine injection – hyperkinesia restored.

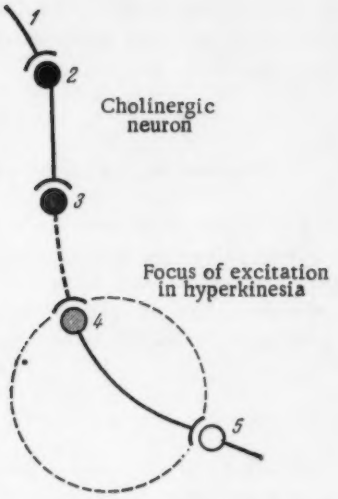

Fig. 2. Diagram showing relationship between cholinergic neuron and neuron whose stimulation leads to development of stem hyperkinesia.

[diphenylacetic acid diethylaminoethyl ester] effect on barbiturate hyperkinesia in rabbits. Scopolamine and methyldiazyl, however, intensified the narcotic effect and shortened the hyperkinetic period. Atropine (0.5, 5 and 100 mg/kg) and methyldiazyl (0.5 mg/kg) were administered to guinea pigs after the development of hyperkinesia. Neither of these drugs eliminated the hyperkinesia.

If atropine was administered first, the subsequent administration of the anticholinesterase agents (0.1-0.2 mg/kg proserine, 1 mg/kg eserine or 0.2 mg/kg phosphacol) failed to inhibit tetridine hyperkinesia even when repeated. A 0.5 mg/kg dose of atropine prevented the effects of anticholinesterase agents in 80-90% of the cases; doses of 5 and 100 mg/kg, in 100% of the cases.

The data presented demonstrate the ability of anticholinesterase agents to inhibit tetridine hyperkinesia. This effect is prevented by the administration of the cholinolytic agent atropine. The diagram in Fig. 2 illustrates the hypothetical relationship between the cholinergic neuron and the neuron which is stimulated in the development of stem (barbiturate and tetridine) hyperkinesia.

Since cholinolytic agents do not prevent or inhibit the hyperkinesia, neuron 4 cannot be cholinergic. Neuron 5 cannot be cholinergic for the same reasons. It is unlikely that the cholinergic neuron immediately precedes the neuron responsible for the hyperkinesia (neuron 3). If neuron 3 were cholinergic, the anticholinesterase agents would intensify hyperkinesia. Therefore, the cholinergic neuron must be located in front of the neuron which, when stimulated, causes hyperkinesia (this is neuron 2 in our drawing, situated one or several neurons above neuron 4), and so neuron 3 is the cholinergic neuron.*

The short duration of the inhibitory effect exerted by proserine, eserine and phosphacol cannot be attributed to their inactivation, since their repeated administration (and consequently, increase of their total dose) gives rise to peripheral effects (fasciculation, salivation, lacrimation, et al.) attending the central effect. It is, however, possible to assume the fixation of these agents by non-specific receptors, as Bovet [2] proposed in the case of succinocholine.

* This paragraph conforms to the Russian original, although it is clearly inconsistent and self-contradictory. It has been suggested that the last sentence should read: ". . .neuron 3 is not the cholinergic neuron," but we have no way of verifying this [Publisher's note].

The fact that phosphacol, which induces an irreversible or hardly reversible inhibition of cholinesterase, behaves in the same way as the reversible inhibitors eserine and proserine deserves special attention. Its interpretation requires investigation of the opinions of several authors [3,10] to the effect that the anticholinesterase and choline-sensitizing effects of this group of compounds should be differentiated. The latter effect is temporary and seems to be paramount in our experiments.

SUMMARY

Experiments were performed on guinea pigs and albino mice. An inquiry was made into the effect of choline-sensitizing and cholinolytic substances on the tetridine hyperkinesis in guinea pigs. Tetridine, as is the case with many barbiturates, apart from the anesthetic effect causes stem hyperkinesis in many animals. Cholinolytics (atropine, scopolamine, tropacine, pentaphene, arpenal, ethyldiazyl and methyldiphacyl) do not prevent tetridine hyperkinesis in mice. Atropine and methyldiazyl do not depress hyperkinesis in guinea pigs. Choline-sensitizing (anticholinesterasic) agents depress tetridine hyperkinesis temporarily. This effect may be repeatedly reproduced in the same animal. Inhibition of tetridine hyperkinesis by a choline-sensitizing agent may be prevented by a cholinolytic substance, such as atropine.

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INHIBITION OF GLUTAMIC-PYRUVIC TRANSAMINASE BY DL-CYCLOSERINE AND OTHER COMPOUNDS

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(Presented by Academician V. V. Zakusov)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 52, No. 7, pp. 76-80, July, 1961
Original article submitted June 11, 1960

In a previous study [1] we showed that D,L-cycloserine at concentrations as low as $2 \cdot 10^{-5}$ M significantly inhibits glutamic-pyruvic transaminase activity in rat liver homogenates. Acyclic precursors of cycloserine—ethyl ester of β-aminohydroxyalanine (KX-9) and β-aminohydroxyalanine (KX-14)—proved to be effective inhibitors as well.

According to the hypothesis of N. K. Kochetkov et al. [2], the free amino group of cycloserine forms with the aldehyde group of phosphopyridoxal Schiff bases which, under mild conditions, can be converted into stable complexes and thus block the enzyme. From this point of view, a necessary prerequisite for blocking of the enzyme is the presence in the inhibitor molecule of a free amino group and a molecular configuration which ensures conversion of the azomethene compound to stable form.

To clarify these matters, we investigated the effectiveness of a number of cycloserine derivatives, and also of certain other compounds *.

Information on the structure of compounds investigated is presented in the table.

METHOD

Experimental conditions and methods were the same as those employed in the preceding study [1]. Glutamic-pyruvic transaminase activity in liver homogenates was determined according to increase in alanine and decrease in pyruvic and glutamic acids. In those cases where inhibitor concentration was below 10^{-3} M, i. e., was 20 times lower than substrate concentration, we disregarded the fact that some of the inhibitors can bind pyruvic acid, or that some are not separated from alanine or glutamic acid during chromatography.

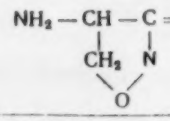
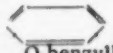
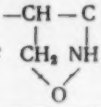
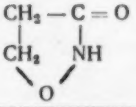
In experiments with higher concentrations, additional control samples were run in which one of the substrates was omitted, and percent inhibition was calculated taking into account the values observed for these samples. Many of the inhibitors bound the carbonyl group of pyruvic acid, thereby removing it from the reaction and being themselves "neutralized". In those cases, we carried out a preliminary incubation of homogenate with inhibitor in order that the inhibitor might act on the transaminase before addition of substrate.

RESULTS

Average figures for 5-6 experiments are presented in the table. They reveal that the acyclic compounds investigated are able to inhibit glutamic-pyruvic transaminase in greater or less degree. In the preceding study [1] we believed it justifiable to assume that the strong effect of KX-9, equal to that of cycloserine, is perhaps related to

* We wish to thank M. Ya. Karpeiskii, R. M. Khomutov, É. I. Budovskii, and E. S. Severin for the gift of compounds synthesized by them, and also B. P. Gottikh for his advice and for the gift of compounds synthesized by him.

Percent Inhibition of Glutamic-Pyruvic Transaminase Activity

Arbitrary designation	Compound	Concentration (moles)								
		10^{-4}	10^{-3}		$2\cdot10^{-3}$		10^{-2}		$2\cdot10^{-2}$	
		-p.	-p.	+p.	-p.	+p.	-p.	+p.	-p.	+p.
	Cycloserine 	100	—	—	—	—	—	—	—	—
KX-9	$NH_2O-CH_2-CHNH_2-COOC_2H_5 \cdot 2HCl$ Ethyl ester of β-aminohydroxyalanine (dihydrochloride)	100	—	—	—	—	—	—	—	—
KX-14	$NH_2O-CH_2-CHNH_2-COOH \cdot HCl \cdot H_2O$ β-aminohydroxyalanine (monohydrochloride)	40	75	—	—	—	—	—	—	—
KX-25	$NH_2O-CH_2-CH_2-CH_2-COOC_2H_5 \cdot HCl$ Ethyl ester of γ-aminohydroxybutyric acid (hydrochloride)	—	60	—	100	—	—	—	—	—
KX-24	$NH_2O-CH_2-CH_2-CH_2-COOH$ γ-aminohydroxybutyric acid	—	30	—	100	—	—	—	—	—
O-BHA*	 O-benzylhydroxylamine(hydrochloride)	—	—	—	36	—	—	100	—	—
KX-10	 N-methylcycloserine	—	—	35	0	—	80	90	—	—
AH-2	 Isoxazolidone-3	—	20	23	—	—	60	—	—	80
AH-1*	$CH_3-CHNH_2-CO-NHOH$ Hydroxamic acid of α-alanine	—	0	—	—	—	—	—	—	70
AH-3	β-chlorpropionhydroxamic acid	—	—	40	—	—	—	—	—	92
Isoniazide*	Hydrazide of isonicotinic acid	—	—		—	—	—	20	—	46

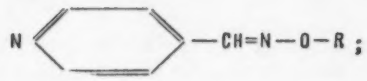
Note: –p.–without preincubation of enzyme and inhibitor; + p. – with preincubation.

* Strongly binds pyruvic acid.

its ability to cyclize relatively easily with formation of cycloserine. It follows from the data of M. A. Breger obtained in the Institute of Pharmacology and Chemotherapy, however, that this compound has a considerably weaker effect on the growth of Mycobacterium tuberculosis bovin 8 than does cycloserine; this casts doubt on the possibility of cyclization of KX-9 to cycloserine.

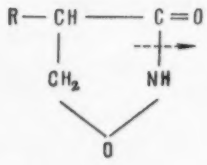
It was also considered possible that pH affects cyclization of this compound. In special experiments it was found, however, that percent inhibition is practically the same at pH 6.5 and pH 7.5. These observations, taken in conjunction with the fact that other acyclic compounds (KX-14, KX-24, KX-25) are also effective inhibitors of transamination, gave reason to believe that KX-9 as such is able to inhibit glutamic-pyruvic transaminase.

Of great interest are the findings that compounds lacking an amino group, but containing a hydroxyamino group, are likewise effective inhibitors (KX-24, KX-25, and O-BHA). If it is granted that the hydroxyamino group of these compounds reacts with the aldehyde group of phosphopyridoxal, obviously a compound of the following type must be formed:



this must be fairly stable in order to block the enzyme.

KX-10 and AH-2, compounds which contain neither a free amino group nor a hydroxyamino group, also possess definite inhibitory activity. If the activity of these compounds is explained in terms of Kochetkov's hypothesis [2], it must be assumed that the ring is ruptured during interaction with the enzyme to free some grouping capable of combining with the aldehyde group of phosphopyridoxal. It is theoretically possible that the ring of isoxazolidone-3 (AH-2), as well as that of N-methylcycloserine (KX-10), is ruptured hydrolytically between the amino nitrogen and the carbonyl carbon:



A rupture of this type must certainly yield a hydroxyamino compound which, as our data show, is an effective inhibitor of glutamic-pyruvic transaminase. Against this assmuption, however, is the fact that no binding of pyruvic acid could be detected when AH-2 was incubated together with enzyme and pyruvic acid, a reaction which must have occurred if a hydroxyamino group were actually formed.

It is of interest that in two cases (see KX-9 and KX-14, and also KX-25 and KX-24) the ethyl esters of acids proved more effective than the acids themselves. It is at present difficult to explain this in any way.

In connection with the considerations of Metzler et al. [7] concerning the role of heavy metal ions in transamination, the observation of N. K. Kochetkov et al. [2],that cycloserine and also a number of its azomethene derivatives are able to form complexes with heavy metal ions,is of interest. This is also true of isoniazide. Cymerman-Craig et al. [4] found a parallelism between the capacity of a number of substituted isoniazides to form such complexes and their capacity to suppress growth of tuberculosis bacilli. It has been suggested that complex formation may prevent normal transamination. This is belied by the finding, however, that EDTA does not inhibit enzymatic processes involving phosphopyridoxal [3,8]. It should be noted, though, that in non-enzymatic transamination EDTA disrupted the metal-phosphopyridoxal-glycine complex [6]. Matsuo believes that the absence of an EDTA effect in enzymatic reactions may be explained by the presence of an apoenzyme which stabilizes the complex of metal with Schiff bases.

In view of these considerations, it seemed to us of interest to determine the effect on glutamic-pyruvic transaminase of hydroxamic acids, which are capable of complex formation. We tested two hydroxamic acids, of which AH-1 may be considered a geometric analogue of cycloserine, and AH-3 a precursor of isoxazolidone. The table shows that both compounds were effective inhibitors, AH-3, with no free amino group, being even more

effective than AH-1, which possesses this group. Thus, all the compounds tested, with the exception of KX-9, proved considerably less effective than cycloserine, but more effective than the extensively used inhibitor of transamination, isoniazide.

From their study of possible mechanisms involved in isoniazide inhibition of transamination, Hicks and Cymerman-Craig [5] concluded, nevertheless, that in all probability the hydrazide group of isoniazide interacts with the aldehyde group of phosphopyridoxal.

Our investigations show that compounds lacking groupings capable of interacting with the aldehyde group of phosphopyridoxal are even more effective inhibitors than isoniazide. It is difficult to believe that the mechanism of inhibition is in principle the same for so strong an inhibitor as cycloserine and so weak a one as isoniazide, while it is different for inhibitors of intermediate effectiveness. Such another mechanism must be postulated for hydroxamic acids, in any case, if it is assumed that the rings of N-methylcycloserine and isoxazolidone-3 can be ruptured. The existence of complex-forming properties in the majority of compounds investigated provides some basis for the supposition that such properties determine the effect of these compounds on glutamic-pyruvic transaminase.

These findings on the effect of various compounds, which may or may not be able to form Schiff bases, indicate that transaminase inhibition may occur by some means other than that involving interference with the reaction of phosphopyridoxal with amino acid substrate.

SUMMARY

The author studied changes in glutamic-pyruvic transaminase activity of liver homogenates under the effect of various compounds capable and incapable of forming Schiff bases with phosphopyridoxal.

The following compounds proved effective in inhibiting the glutamic-pyruvic transaminase activity in the homogenates of rat liver: 1) acyclic-containing both amino and hydroxyamino groups, or hydroxyamino group alone (β-aminohydroxyalanine and γ-aminohydroxybutyric acid and their ethyl esters); 2) cyclic-differing from cycloserine by the absence of a free amino group (N-methylcycloserine and isoxazolidone-3); 3) hydroxamic acids (hydroxamic acid of α-alanine and β-chlorpropionhydroxamic acid). All these compounds, except for the ethyl ester of β-aminohydroxyalanine, the efficacy of which equals that of cycloserine, are less effective than cycloserine; their efficacy, however, is greater than that of isoniazide. Possible mechanisms of action of the above inhibitors are discussed.

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ON THE IMMUNOLOGICAL CHARACTERISTICS OF SERUM PROTEINS

COMMUNICATION II. DETERMINATION OF THE CONTENT OF PROTEIN FRACTIONS IN THE GLOBULIN COMPOSITION IMMUNOLOGICALLY SIMILAR TO ALBUMINS

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 52, No. 7,
pp. 81-83, July, 1961
Original article submitted July 23, 1960

In a previous report [6] we showed that when the α-globulins were isolated by the use of starch block electrophoresis, protein fractions migrated with them which were similar to the albumins in their anaphylactogenic properties. S. Ya. Kaplanskii and co-workers [4] have pointed out the impossibility of obtaining pure antisera to the α-globulins because such antisera yield cross-over serological reactions with the albumins. Using the method of immunoelectrophoresis, traces of albumins were even demonstrated in the composition of purified γ-globulin preparations [3]. These data support the theory of immunological non-uniformity in the individual electrophoretically pure fractions of the serum proteins.

We considered it of interest to determine whether the cross-over serological reactions were a result of inadequate homogeneity of the α-globulins migrating along the path of the albumins in electrophoresis. It is known that the inert carriers used for preparative electrophoresis possess, to a varying degree, a non-specific protein adsorption pattern. However, the possibility is not excluded that a given percent of the α-globulins, possessing a certain similarity to the albumins in their physico-chemical properties [8], are also similar to the latter in their antigenic properties.

METHOD

One group of rabbits was immunized with electrophoretically pure fractions of human serum albumins, isolated by starch block electrophoresis. For the second group we used whole horse serum. The usual scheme of immunization was used on the animals [7]. With the antialbumin sera obtained we carried out a subsequent series of precipitation reactions, adding increasing concentrations of isolated protein fractions from human serum. The degree to which the isolated fractions of the globulins were immunologically similar to the albumins was determined in the ascending portion and in the optimal zone of the precipitation curve constructed for the albumins [10]. The content of antibodies against each of the isolated protein fractions from the horse sera was established in the equivalent zone of the precipitation curve, since in that zone the precipitate contains approximately 10% of the antigen [2].

The concentration of protein in the solutions and in the precipitate was determined by the method of Lowry [9] in the records of A. E. Gurvich and R. B. Kapner [2]. Calibrated curves were separately constructed for crystalline albumin and the γ-globulins. Micro-electrophoresis of the serum was carried out in a Flynn and De Maio type chamber modified by A. E. Gurvich [1].

RESULTS

The antialbumin sera obtained from the rabbits, both after initial immunization and after numerous reimmunizations, invariably yielded cross precipitation reactions with the isolated α-globulin fractions. As can be seen from Table 1, from 10 to 30% of the proteins similar to the albumins in immunological properties migrated in the composition of the α-globulins. It should be noted that in these experiments we used only those fractions of the globulins which did not show observable contamination with proteins of albumin mobility in paper electrophoresis. As a control we used concentrated solutions of globulins (7-10 mg/ml), so as to disclose possible albumin contami-

TABLE 1. Concentration of Protein Fractions Immunologically Similar to Albumins in the Globulins (Average of 3 analyses, performed May 4-10th, 1960, rabbit No. 30)

Rabbit antiserum to human albumin		Antigen			Precipitate	Concentration of protein immunologically similar to albumins (in percent of the total amount of protein in the given fraction)
Concentration of antibodies (in mg/ml)	Volume (in ml)	Globulin fraction of human serum	Concentration (in mg/ml)	Volume (in ml)	Protein concentration (in mg/ml)	
3.10	0.15	α_1	0.45	0.15	1.05	33.3
3.10	0.15	α_2	0.75	0.15	1.39	24.0
3.10	0.15	α_3	0.45	0.15	0.58	12.2
3.10	0.15	β	0.90	0.15	0.30	2.7
3.10	0.15	γ	0.80	0.15	0.12	1.2

TABLE 2. Concentration of Precipitins to the Individual Protein Fractions Subsequent to Immunization of the Rabbits with Whole Serum (Rabbit No. 13, performed October 14-15th, 1959, average of 3 analyses)

Serum fractions	Horse serum-antigen			Antiserum	Precipitate	
	Concentration (in %)	For precipitation				
		Protein concentration (in mg/ml)	Volume (in ml)	Volume (in ml)	Protein concentration (in mg/ml)	Concentration of antibodies (in mg/ml)
Albumins	42.3	0.30	0.15	0.15	0.48	0.43
Globulins:						
α	14.8	0.70	0.15	0.15	1.60	1.44
β	18.3	0.70	0.15	0.15	2.05	1.85
γ	24.6	0.80	0.15	0.15	1.95	1.76

nants, found by the immunochemical method.

Gamma-globulins drawn by an endoosmotic current to the cathode from the site of application of the serum produced an extremely small precipitation reaction with the antialbumin sera. Various samples of the γ-globulins contained approximately 1% of proteins immunologically similar to the albumins. A somewhat higher percent concentration of these proteins was seen in the composition of the β-globulins (an average of 2.5%), although in certain experiments we set up conditions of starch electrophoresis where the β-globulins migrated to the cathode from the site of application of the serum. In this manner it was possible to exclude the influence of the "trailing effect" of the albumins.

The question arises as to why the antisera to the β-and γ-globulins do not yield cross precipitation reactions with the albumins, since these preparations contain minimal amounts of protein which is immunologically similar to the albumins. The purity of these antisera was ascertained several times by S. Ya. Kaplanskii and co-workers. Apparently, when the rabbits are immunized with two or more antigens, the immunological reaction of the organism is dependent upon the strength and concurrent proportion of the different antigens. As can be seen from Table 2, with immunization of the rabbits, using normal horse serum in which the concentration of the albumins and globulins is approximately the same, the production of precipitins against each protein fraction was different. In this case the amount of antibodies to the albumins was always 3-4 times lower than to each of the globulin fractions individually. Naturally, the minimal amount of protein found in the composition of the β and γ-globulins which is immunologically similar to the albumins does not cause a marked production of antibodies to the albumins.

The results obtained by us might be of interest in explaining the mechanism of the combined reactions of serum albumins and globulins in a whole series of diseases. Despite the different pathogenesis of many illnesses (excluding cirrhosis of the liver, multiple myeloma and certain other illnesses), a uniform reaction may be noted

as regards the fractional composition of the serum proteins: hypoalbuminemia with simultaneous increase in the concentration of the α-globulins, and sometimes also the β-globulins. Wouldn't it appear that this increase in the concentration of the globulins is a result of an increase in the production of that protein fraction which is immunologically similar to the albumins ? One encounters evidence in the literature that restoration of the plasma proteins following heavy blood losses in dogs is accompanied by a rapid increase in the concentration of the α_3-globulins, entering into the blood circulation in proportion to the albumin deficit [5]. In this case the α_3-globulins appear to be albumin substitutes [8].

SUMMARY

Isolation of individual fractions of serum proteins was carried out by means of starch block electrophoresis. As shown by the immunochemical quantitative method, a fraction of proteins immunologically similar to albumins is present in the globulin composition. It averaged 30% in the α_1-globulins, 22% in the α_2-globulins, 10% in α_3-globulins, 2.5% in the β-globulins, and 1% in the γ-globulins.

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ON THE SPECIFIC ANTIGEN OF HUMAN SARCOMAS

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 52, No. 7,
pp. 84-89, July, 1961
Original article submitted July 15, 1960

Numerous experimental investigations on the antigenic properties of malignant neoplasms, performed by Soviet scientists over the last 10-15 years, have widened our concepts of the antigenic structure of tumor cells considerably. However, fundamental attention has thus far been paid to study of the antigenic properties of carcinomatous tumors, while the antigenic structure of sarcoma cells has remained almost completely uninvestigated.

It is known that sarcomas are differentiated into the grouping factors A, B and O, just as the erythrocytes and tissues of the same individual [5]. Up until now the presence of species-specific antigens and the type factors M, N and Rh has not been established in sarcomatous tumors. There is also very little data relevant to the specific characteristics of the antigenic structure of sarcomas. Former attempts to demonstrate the so-called sarcoma-specific antigens [10,11] can hardly be considered successful. Recently, using the method of anaphylaxis with desensitization, it was shown that antigens are contained in sarcomas which are absent from the muscle and connective tissue of the embryo and adult human [1,9].

The present investigation was carried out with the purpose of detecting a specific antigen in human sarcomas and of studying its resistance to boiling and to treatment with formalin. In addition, our task was a comparative study of carcinomatous and sarcomatous tumors, in order to cast some light on the question of a similarity or a difference in their specific antigenic properties.

METHOD

As material for the investigation we used 2 sarcomas, removed during surgery, 8 carcinomas, taken from patients posthumously, and, as controls, healthy tissue from the liver, kidney, spleen, heart, muscle, stomach and brain. The fresh tissue was preserved in a refrigerator at -20°, and used as needed. A portion of the tumor material from each case was preserved in formalin (1 part 40% formalin plus 7 parts water). The antigens were prepared from untreated tissue, formalinized tissue, and tissue boiled for 30 minutes. The technique by which the antigens were prepared, and also the procedure for setting up the complement fixation and absorption reactions used in this work, have already been described by us in previous reports [3,4,7]. Following the method which we worked out [6], we prepared specific sera against each of the carcinomas being studied. This method was used to obtain sera against the antigens from sarcomatous tissue. Organ-specific sera against the liver and spleen of a healthy human [8] served as the control. Immunization was performed with antigens from the untreated tissues.

RESULTS

Preliminary experiments for the purpose of studying the character of the antibodies in the immune antisarcoma sera with antigens from sarcomas and a number of normal human organs (liver, kidney, spleen, heart muscle, stomach and brain) showed that the most non-specific binding is seen with the antigens from the liver and spleen. We selected these tissues as adsorbents in the acquisition of specific antisarcoma sera, as well as for the control in all further work. By means of absorption of the immune serum with the formalinized tissue of the normal spleen we obtained specific serum to one of the sarcomas in our possession. An attempt to prepare serum specific for the second sarcoma was unsuccessful, due to an insignificant difference in the titer of the specific and non-specific antibodies in the original immune serum.

TABLE 1. Comparative Study of the Antigenic Properties of Carcinomatous and Sarcomatous Tissues, Based on the Complement Fixation Reaction

Serum	Serum dilution	Antigen from formalinized carcinoma No. 48	Antigens from untreated tissues of sarcoma № 1	Antigens from untreated tissues of sarcoma № 2	Antigens from untreated tissues of a healthy human: Liver	Antigens from untreated tissues of a healthy human: Spleen
No. 991, against sarcoma No. 1	1:20	—	++++	—	—	—
	1:40	—	++++	—	—	—
	1:80	—	++++	—	—	—
	1:160	—	+++	—	—	—
No. 212, against carcinoma No. 48	1:20	++++	—	—	—	—
	1:40	++++	—	—	—	—
	1:80	+++	—	—	—	—
	1:160	++	—	—	—	—
No. 851, against the liver of a healthy human	1:10	—	—	—	++++	—
	1:20	—	—	—	++++	—
	1:40	—	—	—	+++	—
	1:80	—	—	—	++	—
No. 834, against the spleen of a healthy human	1:40	—	±	—	—	++++
	1:80	—	—	—	—	++++
	1:160	—	—	—	—	++++

Arbitrary symbols: ++++, +++, ++, + represent the varying degree of the positive reaction; ± represents a doubtful reaction; — represents a negative reaction.

In Table 1 we present the results of the comparative study on the complement fixation reactions of antigens from the tissues of sarcomas Nos. 1 and 2, carcinoma No. 48, and the liver and spleen of a healthy human with specific sera against the carcinomatous and sarcomatous tumors and organ-specific sera against the liver and spleen.

The clearly manifested positive reaction of the antigen from sarcoma No. 1 with the antisarcoma serum No. 991, in the presence of a negative reaction of that serum with antigens from the spleen and liver, presents a convincing argument that a specific antigen is contained in the human sarcoma which is absent from healthy tissues (in this case, the liver and spleen). The specificity of the reaction is confirmed by the results of the interaction of these antigens with the organ-specific sera Nos. 851 and 834.

Multiple trials of the antigen from the second sarcoma yielded negative results for the complement fixation reaction with the sera obtained by us, as is seen in Table 1. On the basis of this it may be postulated that sarcomas of different localization and structure may contain different specific antigens.

In the comparison of the specific antigenic properties of carcinomatous and sarcomatous tumors we observed that the sarcoma antigen does not react with any of the 8 anticarcinoma sera, just as the antigens from the carcinomas studied by us did not react with the serum against sarcoma. From the data in Table 1 it follows that anticarcinoma serum No. 212 fixes complement only with the antigen from carcinoma and does not react in the presence of extracts from sarcoma No. 1, No. 2 or from the control tissues. At the same time, antisarcoma serum yields a positive reaction with the antigen from sarcoma, but, as has already been noted, does not react with the antigen from carcinomatous tumor. The results of these trials testify to a difference in the specific antigenic properties of carcinomatous and sarcomatous tissues in the human. Carcinomas contain a specific antigen, absent from the sarcoma, and, on the other hand, sarcoma cells contain their own specific sarcoma antigen, which is not encountered in carcinomas.

Besides the complement fixation reaction, a comparison of the specific tumor antigens was carried out in absorption experiments, absorbing the antitumor sera with untreated cells from the carcinoma and sarcoma. Despite inadequate clarity of the obtained results, due to the anticomplementary properties of the sera following absorption,

TABLE 2. The Effect of Formalin and Boiling on the Antigenic Properties of Sarcoma and Carcinoma

Serum	Serum dilution	Antigens from the tissues							
		of carcinoma No. 92			of sarcoma No. 1			normal and untreated	
		untreated	formalinized	boiled	untreated	formalinized	boiled	liver	spleen
No. 991, against sarcoma No. 1	1:20	—	—	—	++++	—	—	—	—
	1:40	—	—	—	++++	—	—	—	—
	1:80	—	—	—	++++	—	—	—	—
	1:160	—	—	—	+++	—	—	—	—
No. 211, against carcinoma No. 92	1:40	++++	++++	++++	±	—	—	—	±
	1:80	++++	++++	++++	—	—	—	—	—
	1:160	++++	++++	+++	—	—	—	—	—
	1:320	++	++	++	—	—	—	—	—
No. 834, against healthy human spleen	1:40	—	—	—	+	±	—	±	++++
	1:80	—	—	—	—	—	—	—	++++
	1:160	—	—	—	—	—	—	—	++++
	1:320	—	—	—	—	—	—	—	++

Arbitrary symbols the same as in Table 1.

an antigenic difference was again indicated between the carcinomatous and sarcomatous tissues.

We have collected data on the impressive stability of the specific antigens from the carcinomas against the action of certain physico-chemical factors, such as freezing, drying, high temperature, and formalin [2,4].

In tests of the antigens from sarcomatous tissue subjected to preliminary boiling for 30 min or treatment with formalin, we discovered, using the complement fixation test, that the antisarcoma serum does not fix complement in the presence of antigens from sarcomatous tissue preserved in formalin or heated for 30 min at 100°, while there is a clearly manifested positive reaction between this serum and the antigen from the untreated sarcoma tissue. This can be seen from the data in Table 2. On the other hand, aqueous-saline extracts of carcinomas, both from the untreated as well as the tissues treated with formalin or heating, yield a positive reaction for fixation with the corresponding anticarcinoma serum No. 211.

Thus, the resistance of the specific antigens from the carcinoma and sarcoma cells to the action of certain physico-chemical factors is not the same.

The experiments showed that the specific sarcoma antigen, in contrast to that of carcinoma, is destroyed by boiling or treatment of the tissue with formalin. These data were confirmed by absorption of the serum on tumor cells subjected to the action of formalin or boiling.

In Table 3 we present the protocol of the absorption experiment, involving antisarcoma (No. 991) and anticarcinoma (No. 204) sera absorbed on the cells of formalinized sarcoma and carcinoma. In the first horizontal column we show the reaction of each of the sera with the test antigens before absorption. In the remaining four are reflected the results following the interaction of the sera with the tumor cells. From the data in Table 3 it is apparent that absorption of the antisarcoma serum by the cells of the formalinized carcinoma or sarcoma did not deprive it of its ability to enter into reaction with the antigen from untreated sarcoma tissue. In other words, the sarcoma or carcinoma cells treated with formalin did not extract the specific antibodies from the antisarcoma serum. This may be explained by the fact that these tissues did not contain the antigen specific for sarcoma: in the carcinoma it is absent, and in the sarcoma it is inactivated by formalin. As a result of absorption of the anticarcinoma serum with homologous tissue, i. e. carcinoma, a complete extraction of the specific antibodies occurred. When the second portion of this serum was treated with sarcoma tissue preserved in formalin, the reaction with the carcinoma antigen was not lost.

TABLE 3. Absorption Studies of the Antigenic Properties of Sarcoma Treated with Formalin

Serum	Formalinized tissue, used for the absorption of the serum	Serum dilution	Antigens from the tissue: of formalinized carcinoma No. 48	of untreated sarcoma No. 1	of formalinized sarcoma No. 1	of untreated healthy human spleen
No. 991, against sarcoma No. 1	—	1:20	—	++++	—	—
		1:40	—	++++	—	—
		1:80	—	++++	—	—
	Carcinoma No. 48	1:20	—	++++	—	—
		1:40	—	++++	—	—
		1:80	—	++++	—	—
	Sarcoma No. 1	1:20	—	++++	—	—
		1:40	—	++++	—	—
		1:80	—	++++	—	—
No. 204, against carcinoma No. 48	—	1:20	++++	—	—	—
		1:40	+++	—	—	—
		1:80	+	—	—	—
	Carcinoma No. 48	1:20	—	—	—	—
		1:40	—	—	—	—
		1:80	—	—	—	—
	Sarcoma No. 1	1:20	++++	±	—	—
		1:40	+++	—	—	—
		1:80	+	—	—	—

Arbitrary symbols the same as in Table 1.

TABLE 4. Absorption Study of the Antigenic Properties of Sarcoma Following Boiling

Serum	Tissue used for the absorption of serum	Serum dilution	Antigens from the tissues: of untreated sarcoma No. 1	of sarcoma No. 1 following boiling	of untreated healthy human spleen
No. 991, against sarcoma No. 1	—	1:20	++++	—	±
		1:40	++++	—	—
		1:80	+++	—	—
	Sarcoma No. 1 after boiling	1:20	++++	—	—
		1:40	++++	—	—
		1:80	+++	—	—
No. 834, against healthy human spleen	—	1:20	—	—	+++
		1:40	—	—	++
		1:80	—	—	±

Arbitrary symbols the same as in Table 1.

The absorption experiments showed that not only extracts but even the sarcoma cells thenselves lose their specific antigenic properties when treated with formalin.

The data obtained in the absorption experiments with antisarcoma and anticarcinoma sera absorbed upon tumor cells subjected to boiling for 30 min correspond to the results of the experiments with the formalinized cells.

As an example, in Table 4 we present the protocol of an absorption experiment involving antisarcoma serum absorbed upon previously boiled sarcoma cells.

From the data in Table 4 it follows that serum does not lose its specific antibodies after treatment with cells from a sarcoma exposed to heat, which can be discerned from the positive reaction of the serum with untreated antigen from the sarcoma.

Our investigations indicate that a specific antigen is contained in the sarcoma cells which is absent from carcinoma and normal human tissues. The specific sarcoma antigen, in contrast to that of carcinoma, is destroyed by boiling or treatment of the tissue with formalin.

SUMMARY

The presence of specific antigen was established in the crude tissue of human sarcoma by immunological methods of investigation. This antigen differed from those of the normal tissues and of the human cancer tumors. A comparative study of some physico-chemical properties of specific antigens of cancer and sarcomatous tumors has demonstrated that the sarcoma specific antigen, as distinct from the cancer one, disintegrates during boiling or formalin treatment of the tissue.

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THE ROLE OF THE LYMPH NODES IN ACQUIRED ANTITUMOR IMMUNITY

COMMUNICATION I. CHANGES IN THE REGIONAL LYMPH NODES FOLLOWING HOMOTRANSPLANTATION OF THE BROWN-PEARCE TUMOR

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 52, No. 7, pp. 89-95, July, 1961
Original article submitted April 25, 1960

A considerable body of experimental data has accumulated in the field of immunology, indicating that the antibody is formed in certain cells of the lymphoid tissue [6,8,13]. These cells are considered to be young cellular elements of reticular origin, belonging to the lymphoid-plasmocyte series and known under the names: immature plasma cells [13], plasmoblasts and lymphoblasts [2,3], large lymph cells [19], etc. Immunomorphological changes are fundamental, lying at the root of the production of antibodies and their entrance into the blood circulation.

These data are of major interest in explaining the mechanism of acquired immunity to tumors, since there is no adequate basis for explaining it as a result of humoral antibodies [5,7]. A number of factors speak for a direct relationship between the lymph tissue and the immune antitumor processes; these became known as a result of a histological study of the cellular reaction about the periphery of a tumor [1,12,15], through experiments on the antiblastic effect of lymph tissue in vitro [14,20], and by experiments on the passive transfer of antitumor immunity using cells from regional lymph nodes [18].

In connection with these data the question arose as to whether the process of the formation of antitumor immunity was reflected in morphological changes in the lymph tissue. Only isolated reports exist in the literature on an increase in weight of regional lymph nodes in animals with tumor homotransplants [9,10]; occasional references have been made to certain cellular changes in these nodes and in the spleen—lymphoid hyperplasia and plasma cell reaction [11,15,17]. We were not able to find any descriptions in the literature of the dynamics of the cell changes with development of antitumor immunity.

In our work we attempted to investigate the state of the lymph nodes during the growth and resorption of a tumor. For this purpose we used the schema: homotransplantation of the Brown-Pearce tumor to the helix of the ear in rabbits, a method worked out by us earlier [4]. This method is very suitable for resolving the proposed question, thanks to its following characteristics: with transplantation to the helix the development of the tumor attains a strict cyclic character; its active growth regularly alternates with resorption in comparatively uniform time intervals in the different animals. Resorption of the tumor node insures the development of effective immunity against the second transplantation of the same tumor. We could not detect an antitumor antibody in the blood of the immune rabbits [5]; thus, it was especially desirable to perform an immunomorphological investigation. Finally, the helix of the ear in the rabbit has well-developed regional lymph nodes.

In this report we present the results of an investigation of the lymph nodes at various stages in the growth and resorption of a tumor homotransplant.

METHOD

The investigation was carried out on rabbits of the chinchilla family, using both sexes. The Brown-Pearce tumor was transplanted to the rabbits by the method previously adopted [4]: a 0.28-0.3 ml 40% suspension of fresh tumor tissue was injected subcutaneously into the helix. In each case we made a quantitative appraisal of the tumor's developmen-

tal state, based on measurement of its area at the maximum cross section. Following transplantation, the rabbits were sacrificed at various stages of the tumor's development: in a growth period– on the 1st, 3rd and 6th days, and a resorption period– on the 10th, 15th and 34th days, which corresponded to the beginning, middle and end of tumor resorption (Table 1). As a control we sacrificed 8 normal rabbits, to whom no tumor was administered.

TABLE 1. Changes in the Weight of the Regional Lymph Nodes During the Development of the Brown-Pearce Tumor in the Helix of Rabbits (Mean Values)

Group of rabbits	Number of rabbits	Period of tumor development	Days following transplantation	Tumor measurement (in cm^2)			Total weight of the three nodes (in mg)			Weight of node No. 1 (in mg)		
				maximum	On the day animal was sacrificed	Percent decrease	Regional nodes	Contralateral nodes	Degree of increase	Regional node	Contralateral node	Degree of increase
First	8	Control rabbits					143	143	1.0	50	51	1.0
Second	4	Growth	1	-	-	-	229	219	1.0	89	72	1.2
Third	4	"	3	-	-	-	280	143	2.0	138	47	2.9
Fourth	6	"	6	-	1.45	-	333	119	2.8	163	39	4.2
Fifth	5	Resorption	10	2.35	2.11	10.2	470	231	2.1	-	-	-
Sixth	7	The same	15	2.35	1.35	42.5	670	308	2.1	205	64	3.4
Seventh	5	"	33	2.29	0.37	83.8	258	201	1.2	103	71	1.2

By injecting India ink into the helix in preliminary experiments, it was established that the latter has three regional lymph nodes. The basic lymph flow was to a large node in the root of the helix, which we arbitrarily designated No. 1. In each rabbit we extracted three lymph nodes on the side of the tumor (regional), and three nodes on the opposite side (contralateral). After they were freed from the surrounding fat tissue, the nodes were subjected to careful examination and weighing.

Both the total weight of the three nodes and the individual weight of node No. 1 were noted, since the latter underwent the greatest increase in size.

For the morphological investigation we used prepared cytological samples, which were stained with azure-II-eosin. Node No. 1 was subjected to cytological investigation with a count of the basic cell forms, inasmuch as the changes in it were reflected the most clearly. For each node the cells were counted in 60 fields, 30 fields in each of two samples (objective ×90, ocular ×7, binocular eyepiece). For each of the cell forms in these two counts we took the average number for thirty fields, which corresponded to an average of 3500-4000 cells in total. Special attention was directed to changes in the content of reticular cells, lymphoblasts and plasmocytes; the corresponding data were subjected to statistical analysis. The lymphocytes and prolymphocytes, always composing more than 90% of the cells, were not counted.

RESULTS

The data from the macroscopic appraisal of the lymph nodes are presented in Table 1 and in Fig. 1. Development of the tumor in the helix occurred in the following manner: in all the rabbits the tumor survived the transplantation and showed a cyclic character of development. Hyperemia and edema were observed at the site of injection 24 hours after the inoculation. On the 3rd day the inflammatory signs decreased and it was possible to recognize the beginning of tumor growth. On the 6th day the tumor already showed large dimensions and looked like a large formation of oval form. The tumor nodule reached its maximum size on the 8th day in the majority of the animals. Then the tumor began to decrease in dimensions: by an average of 10.2% on the 10th day, and almost half by the 15th day. In a large part of the rabbits the resorption was finished after 3-4 weeks. Thus, when transplanted to the helix, the fate of the homotransplant from the Brown-Pearce tumor inoculate remarkably recalls the fate of a normal tissue transplant, both in the constancy of its developing regression and in the time intervals and suddenness of its onset [4].

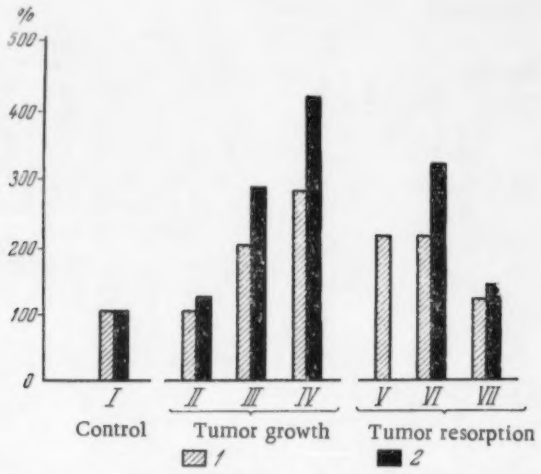

Fig. 1. Changes in the weight of the regional lymph nodes during the development of the Brown-Pearce tumor in the helix of rabbits (in percents of the weight of the contralateral nodes, the latter taken as 100%). I-VII: rabbit groups. 1) Total weight of the three nodes; 2) weight of node No. 1.

Investigation of the regional lymph nodes showed that during the period of tumor development they undergo profound changes, the nature of which is closely linked to the stage of the tumor process.

From Table 1 it can be seen that one day after the tumor transplantation the nodes on the experimental side did not differ from the controls in weight. In vivo palpation begins to detect an increase in node No. 1 on the 2nd day; on the 3rd day the nodes on the tumor side are already markedly increased, and their weight exceeds the weight of the nodes on the contralateral side by more than 2 times. Node No. 1 undergoes the greatest increase. Maximal hypertrophy of the nodes is attained on the 6th day following transplantation, the day before the tumor ceases to grow. The altered nodes markedly increase in volume, and appear succulent on sectioning. On the first days of the onset of tumor resorption the lymph nodes also begin to decrease, and by the end of resorption the weight of the regional nodes approaches the weight of the contralateral nodes. We were unable to detect any visible changes in the latter throughout the extent of the tumor process.

From this it would follow that the development of the Brown-Pearce tumor homotransplant in the helix of rabbits is accompanied by a markedly demonstrated enlargement of the regional lymph nodes. This enlargement develops from the beginning of tumor growth, and attains its maximum before the tumor stops growing and undergoes regression. As the tumor is resorbed the enlargement of the regional lymph nodes also gradually disappears.

As the microscopic investigation showed, the growth of the nodes is not related to metastasis of the tumor; only in one node were there observed individual tumor cells, undergoing degeneration.

Study of the prepared cytological samples showed that the basis for the hypertrophy lay in the profound changes in cell composition of the nodes (Table 2, Fig. 2).

In the normal rabbits the cytogram for the nodes of both sides was identical. The basic mass of cell elements consisted of lymphocytes and prolymphocytes. The number of lymphoblasts did not exceed 27 per 30 visual fields. There were a large number of reticular cells. The remaining cell elements (polyblasts, mature plasma cells, eosinophiles) were counted individually per 30 visual fields; as a rule, pseudoeosinophiles and macrophages were not encountered.

Even one day after transplantation of the tumor the cytogram for the node on the tumor side differed from the cytogram of the contralateral node. No changes of any kind were detected in the latter. In the experimental node we noted stimulation of the reticuloendothelium, with an increase in the number of reactive cells – polyblasts,

TABLE 2. Cell Changes in the Lymph Nodes During Development of the Brown-Pearce Tumor in the Helix of Rabbits

Group of rabbits	Number of rabbits	Period of tumor development	Day following transplantation	Lymph node	Cell forms: Reticulocytes	Polyblasts	Macrophages	Lymphoblasts	Plasmocytes	Eosinophiles	Pseudoeosinophiles	Mitoses
First	5	Control rabbits		R	111.8	3.6	0	19.1	4.1	6.0	0.2	1.0
				C	107.7	3.9	0.2	22.8	8.1	5.2	0.8	0.4
Second	3	Growth	1st	R	112.8	20.2	1.0	16.4	9.4	2.9	9.0	0
				C	127.2	4.2	0.3	16.7	3.0	1.7	1.5	0.5
Third	4	»	3rd	R	59.7	47.6	5.9	85.1	16.7	3.7	16.1	2.0
				C	122.3	17.5	0.3	20.8	4.2	1.0	2.8	0.2
Fourth	5	»	6th	R	55.5	26.1	4.8	134.4	15.1	0.7	2.7	1.8
				C	112.8	15.5	0.9	24.2	3.9	1.2	1.3	0.4
Fifth	3	Resorption	10th	R	61.2	13.2	2.3	144.5	18.8	2.5	1.3	6.2
				C	120.0	19.8	0.7	37.0	4.8	4.7	2.3	0.2
Sixth	5	The same	15th	R	69.0	10.6	2.4	115.7	16.5	1.8	1.8	2.1
				C	103.9	14.2	0.7	42.6	6.2	1.7	2.1	0.9
Seventh	4	» »	34th	R	105.9	4.7	2.5	49.5	2.5	2.0	1.0	0.6
				C	101.2	4.5	1.1	35.5	3.3	2.6	1.2	0.3

Note. In each column - the average number of cells per 30 visual fields (3500-4000 cells in total); R - regional node; C - contralateral node.

having a reticular derivation. Pseudoeosinophiles and single macrophages were noted.

On the third day following transplantation the changes in the node on the tumor side were more profoundly developed. The decrease in the number of typical reticular cells was striking. However, the other cell forms of reticular derivation were increased in number–polyblasts and macrophages, associated with a well-defined erythrophagia. A small increase was detectable in the number of mature plasmocytes. The most characteristic feature of the microscopic picture was the appearance of new cell forms, having the following traits: primarily large cells of round or oval form, sometimes having pseudopods. A large round nucleus occupied a central or slightly eccentric position, and contained a fine network structure which stained weakly. The cytoplasm was markedly basophilic and often vacuolated. Mitotic figures were occasionally seen in these cells.

The described cells are apparently of reticular derivation, since transitional forms can be found between them and the typical reticular cells. The morphological characteristics justify placing the described cells in the category of young cells of the hemocytoblast-lymphoblast type. For brevity in designation we unite them into one group, the lymphoblasts.

On the 6th day following transplantation of the tumor the changes in the cell composition of the regional node reached full development. Most characteristic was a further increase in the number of cells of the lymphoblast type. Proliferation of these cells occurred throughout the entire node. In the cortical layer these cells sometimes suggested plasmoblasts (Fig. 3), but we refrained from using this name since from these cells we trace the gamma transitional forms to the typical prolymphocytes, and not to plasmocytes. At this time there is the well defined appearance of reticuloendothelial stimulation and polyblastic reaction in the contralateral node.

In the process of tumor resorption the neogenesis of lymphoblast-like cells continues, but its intensity decreases; the cells of the earlier developmental intervals become smaller, while larger prolymphocytes appear. The number of plasma cells does not increase in comparison with the previous periods. The number of normal reticular cells goes up. A small lymphoblastic reaction can be detected in the contralateral node.

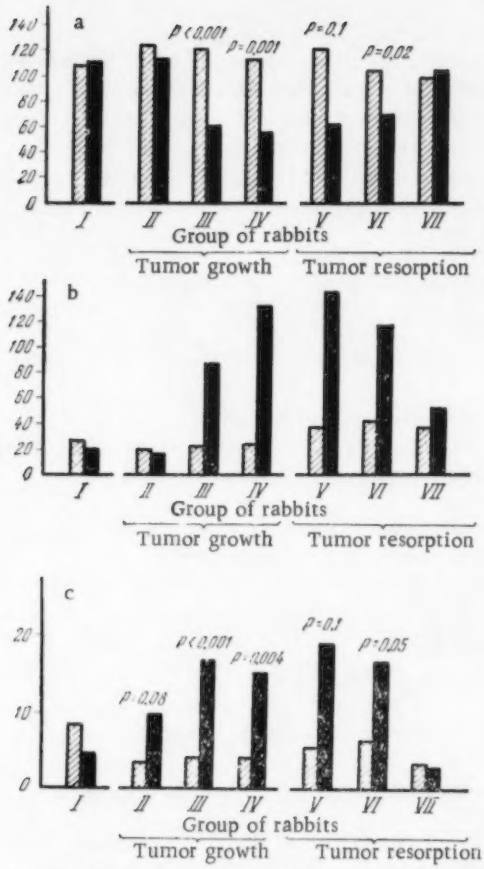

Fig. 2. Dynamics of the cell forms in the lymph nodes during the development of the Brown-Pearce tumor in the helix of rabbits. a) Reticular cells; b) lymphoblasts; c) mature plasma cells; black bar–regional node; cross-hatched bar–contralateral node. On the ordinate axis–number of cells per 30 visual fields (a,b,c).

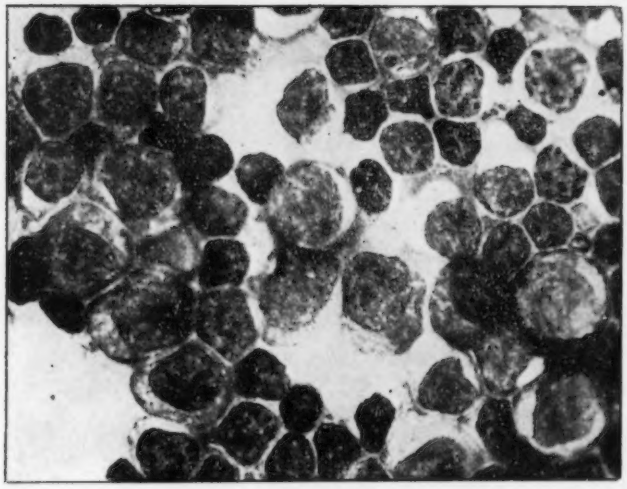

Fig. 3. Cellular reaction in the regional lymph node at the period of its maximal hypertrophy. The large lymphoblast-like cells are numerous. Magnification ×800.

As the tumor resorption approaches the end, all the cellular shifts gradually decrease and the cytogram of the node increasingly approximates the normal.

Thus, the investigation showed that the basis for hypertrophy of the regional lymph node, developing in response to tumor transplantation, lies in the intense proliferation of cellular elements of reticular derivation, belonging, by their cytological characteristics, to the young cell category of the lymphoblast type. This cellular reaction primarily develops in the regional nodes, but it is also noted, to a weak degree, in other lymph nodes. Complete development of the cellular reaction is attained on the 6th day following transplantation of the tumor, i. e., before the tumor begins resorption. During the process of tumor resorption the cell changes gradually disappear.

A comparison of our results with literature data [2,3,13,19] shows that the character of the changes observed, the time intervals of their onset, their dynamics and the site of development are very similar to the cell changes which characterize the process of immunogenesis associated with the action of foreign body antigens. It is important to emphasize that we did not observe a transition of the basic mass of young cell forms to mature plasma cells.

It is of interest to juxtapose that fact with our data showing that during regression of the Brown-Pearce tumor in the helix we could not demonstrate antibody in the blood of the rabbits [5]. The literature records that the appearance of adult plasmocytes is characteristic for the process of hyperimmunization, and observes a parallel relationship between the degree of their formation and the rise in antibody titer in the blood [2,13].

The results of the investigation show that the process of development of antitumor immunity, associated with temporary survival and subsequent resorption of the tumor homotransplant, is vividly reflected by morphological shifts in the regional lymph nodes which are characteristic for the processes of immunogenesis. A deeper study of the immunological nature of these shifts will serve as a project for subsequent experiments.

SUMMARY

The author studied the state of the lymph nodes in dynamics during the growth and resolution of the Brown-Pearce tumor transplant in the helix of rabbits. From the very first days of the tumor growth the regional lymph nodes are subject to considerable hypertrophy, reaching its greatest development prior to the resorption of the tumor. At the basis of hypertrophy there is an intensive formation of cellular elements of reticular origin and those related to the young cells of the lymphoblast type. Cellular reaction develops mostly in the regional lymph nodes and reaches its complete development on the 6th day after the inoculation of the tumor. Cellular shifts are reduced in the process of tumor regression and disappear with its completion.

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THE INFLUENCE OF HIBERNATION ON THE DEVELOPMENT OF EXPERIMENTAL TUMORS IN CITELLUS MAXIMUS PALL.

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 52, No. 7,
pp. 96-98, July, 1961
Original article submitted July 8, 1960

The genesis of malignant neoplasms caused by a solution of 9,10-dimethyl-1,2-benzanthracene (DMBA) was suppressed throughout 4 ½ months of winter hibernation in Citellus suslica Gueldenstaedt [4,5] and the 10-week "summer" hibernation in C. erythrogenis Br. [3]. This influence was seen to be considerably stronger than in hamsters [1,2], since hibernation in the latter is not as deep as in the marmots.

In this work an attempt was made to clarify how a shorter but equally deep "summer" hibernation would affect the course of the same process in marmots of another species. For this purpose we used C. maximus Pall.

METHOD

All the animals were given a single injection of DMBA in a dose of 2 mg in 0.2 ml benzene subcutaneously, into the inner surface of the left thigh. When 72 days had elapsed after the injection all the animals participating in the experiment were divided into 2 groups. One group of 11 marmots, after initially being maintained for three days on dry food material (oats), was placed in a cellar, where the temperature was maintained within the range of 15-16°. The other group, with the same number of animals, remained in the normal animal quarters and served as control. In the course of the first week 3 marmots of the first group, having not entered into the hibernation state, were transferred to the regular quarters and added to the control group. The 8 marmots remaining in the cellar fell into a deep hibernation. At that time their rectal temperature dropped to 16.3-17°, while in the non-hibernating animals there (which were later removed) the temperature was 32-33°. In the marmots of the control group, residing in the normal quarters, the temperature was 33.5-35°.

Fig. 1. Sandstone marmot. Tumor at 2 years and 5 months after the injection of DMBA.

After a two-week period all the marmots were returned to the normal quarters and awakened from their hibernation. Subsequently, no member of that group returned to the deep hibernation. In April, 1957 there remained 7 animals in the experimental group and five in the control, the rest having died. Observations were continued up until the natural death of all the animals. The last marmot died on November 28, 1958. Thus, our observations lasted over a period of 2 ½ years (the injections were carried out on June 12, 1956).

All the marmots were autopsied after their deaths, and the injection site—the skin and underlying tissue—was subjected to histological investigation. We employed the method of imbedding in paraffin and staining with Bemer's hematoxylin, eosin, and by the method of van Gieson.

In the course of the experiment we recorded minimal hair losses temporarily appearing in the marmots of both groups. We never observed neoplasms or pathological changes which could be interpreted as pre-tumors in the group entering the hibernation state.

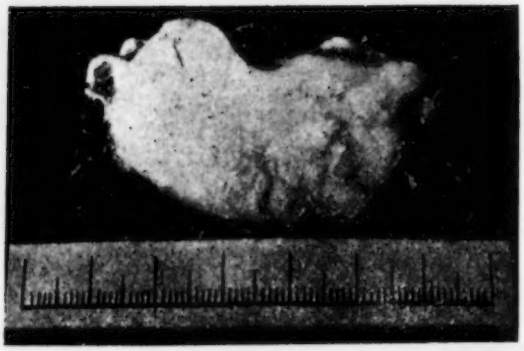

Fig. 2. The same tumor as in Fig. 1, in cross section. Above and to the left can be seen a portion of the spherical node situated on the medial protuberance.

A neoplasm arose in one female in the group of animals that did not fall into hibernation. At the beginning of May, 1958, no pathological changes were observed in her. In the middle of May a thickening appeared, fixed to the underlying tissue, which was located to the left on the posterior portion of the peritoneal wall at the level of the articulation between the femur and the pelvis. The skin over the neoplasm was movable. The thickening had an imperfectly oval nodular form, and a dense consistency. By June 13th, 1958 its diameters were equal to 23 and 18 mm. Gradually, the skin over it became fixed, and the neoplasm, remaining just as dense and nodular, markedly increased in dimensions. By November 18th, 1958 it attained the proportions 50×46×23 mm. In Fig. 1 we see the marmot at that time.

It was now possible to establish that the neoplasm consisted of three fused protuberances. The largest of these was located on the external side and anterior, while the two others—smaller in size—were turned inward and posteriorly. The marmot died on November 28, 1958, 9 days after a biopsy was carried out on it; at that time a small scar remained at the site of the operative wound. Autopsy showed that the protuberances which were felt through the skin on the side of the peritoneal wall were also well defined. To a greater or lesser degree, fluctuation was felt in all three of the protuberances. On the medial protuberance at the level of the linea alba in the peritoneal cavity there was a spherical node of reddish-brown color. Lateral and anterior to this node at one place the mesenteric folds were loosely attached to the peritoneum overlying the tumor, the latter making the entire surface of the tumor appear lustrous. The weight of the tumor was 43 g.

On sectioning it could be seen that there were no borders between the protuberances: the entire tumor appeared as a uniform, dense mass, varying only in color. Its central portion was a grayish-brown color, the entire peripheral (larger) portion was pink, and in the region of the spherical node mentioned before it was a vivid red. A small number of white strands were threaded through the whole tumor; large blood vessels accompanied by parallel running nerves could be seen in places. No metastases were discovered. In Fig. 2 the tumor is presented in cross section (photograph taken after fixation of the material in a 10% solution of formalin).

With microscopic investigation of the sections from various portions of the tumor no essential differences could be found between them. The neoplasm turned out to be a spindle cell sarcoma with a large admixture of polymorphic cells. The tumor tissue contained a significant number of dilated thin-walled blood vessels. Mitotic figures were seen everywhere. Fibers, staining a pink color by van Gieson's technique, were present in very small number. The tumor cells infiltrated and destroyed the adjacent muscle and subcutaneous fat tissue, penetrating into the dermis. A portion of the tumor, with isolated adipose tissue cells retained, is presented in Fig. 3. Sites of necrosis were encountered in some areas of the visual field.

Biological proof of the malignancy of this neoplasm was also provided by the fact that transplantation of portions of it yielded a high percent of "takes", and no tendency toward a decrease in this figure was seen in the course of 4 passages of homo- and heterotransplants.

No final conclusions can yet be drawn from this experiment. However, it can be stated, in the first place, that we obtained an experimental malignant tumor from a sandstone marmot, a species in which a neoplasm had not yet been described; secondly, our data on the influence of deep hibernation (even of relatively short duration) coincides with previously obtained results from experiments on other species of marmots which enter into a deep hibernation—speckled and red-jawed marmots.

In conclusion, it should be noted that among different families of the rodent order the squirrel family, to which belong the marmots, is characterized by an exceptional infrequency of "spontaneous" tumors and by a low output of experimental neoplasms [3].

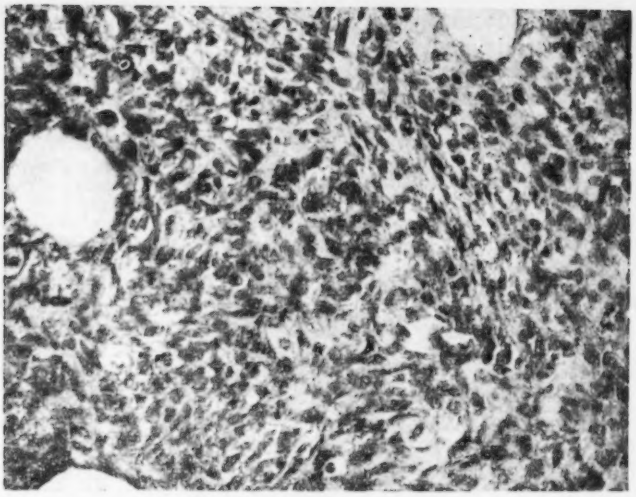

Fig. 3. The same tumor as in Figs. 1 and 2. Sarcoma. Projections of spindle-shaped cells can be seen, along with polymorphic cells, mitoses and remnants of adipose tissue. Magnification 150×.

SUMMARY

A tumor caused by the administration of 9, 10-dimethyl-1,2-benzanthracene developed in one Citellus maximus Pall. from the non-hibernating group in one year and eleven months. Within five months it reached the size of 50×46×23 mm. This neoformation proved to be a spindlecell sarcoma with considerable polymorphism. It is markedly transplantable. No signs of tumor growth were detected in the experimental group of animals which were in a deep summer hibernation for two weeks.

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WAYS OF TRANSPORT OF THE SECRETION FROM THE HYPOTHALAMIC CENTERS INTO THE NEUROHYPOPHYSIS

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(Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 52, No. 7, pp. 99-103, July, 1961
Original article submitted October 12, 1960

The conception according to which a neurosecretory product is transported through the supraoptico-hypophyseal tract in mammals and through its analogue in lower vertebrates—the preoptico-hypophyseal tract from the hypothalamus to the pituitary gland—is based on the fact that along the nerve fibers a Gomori-positive substance can be found, and on the results following the transsection of these fibers [7,10,11,12,16,18]. The neurosecretory substance, which is transported over numerous nerve fibers, is condensed in the region of the median eminence of the infundibulum or in the posterior lobe of the pituitary gland, round the endothelial walls of the numerous blood vessels. These vessels, taken as a whole, represent two portal systems which secure an independent supply of the modified secretion to the anterior and intermediate lobe of the pituitary gland. [4,6,8,9,18]. Part of the nerve fibers from the posterior lobe enter the intermediate lobe of the pituitary gland; in a similar manner nerve fibers from the median eminence and the infundibulum enter, together with the blood vessels, the tuberal part [5,6,19]. According to recent findings in mammals a great number of nerve fibers connect the wall of the infundibulum with the meninges embracing the pituitary gland and its tuberal lobe [15]. Such nerve fibers are absent from the anterior lobe of the pituitary gland.

In recent years an increasing number of authors reported that the hypothalamo-hypophyseal tract does not represent the only way in which the neurosecretory substance can be transported. It could be established that the product of neurosecretion is conveyed toward the oral cavity [5,15]; others reported its transfer into the cerebrospinal fluid of the IIIrd ventricle [8,12,13]. The rich vascularization of the hypothalamic nuclei, the close contact between individual neurons and the endothelium and the numerous capillaries emphasize the possibility of a direct outflow of the neurosecretory substance with the blood. The discrepancy between the quantity of secretion found in the posterior lobe of the pituitary gland in tadpoles and its low concentration in various parts of the preoptico-hypophyseal tract suggests that the neurosecretory substance may be transported in other ways as well [1].

In the present paper we quote new findings concerning various ways of transport of the neurosecretory substance and in particular we discuss its possible transport by wandering cells.

METHOD

Anurans of various age, white rats and puppies served as objects of investigation. Amphibia are interesting in view of the fact that from a phylogenetical point of view their hypophyseal system represents the earliest stage of the formation of the organization so characteristic for all higher vertebrates which is developed to perfection in mammals. The material was fixed in Bouin's liquid, fixative of Shabadash, Zenker-formol and 10% neutral formalin; the material was embedded in paraffin; sagittal and transversal sections were stained with Gomori's hematoxylin with phloxin, aldehyde-fuchsin, asano (according to Heidenhain), and by the method of Halmy as modified by Dyban, and the P.A.S. reaction for polysaccharides was used. Also, the sections were treated by the method of Brachet to demonstrate nucleic acids.

RESULTS

Study of the preoptic region in Rana esculenta and Rana temporaria, the spadefoot toad (Pelobates fucus) and the green toad (Bufo viridis) revealed that the structure of the neurosecretory substance is different [2,3] in various species with regard to the process of secretion and formation. In the toad granules, and in the frog droplets, of varying

diameter predominate; the path of outflow, however, seems to be identical (see Figs. a,b). The preoptic nuclei, which are large and extend over a considerable area, are arranged symmetrically in the walls of the preoptic fossa and can be divided into two parts: the lower elongated ventromedial and the upper, more compact, dorsolateral part. In the lower part of the nucleus the neurosecretory substance, which gives a positive staining reaction, extends along the axons or the ependymal processes, in the shape of granules or spheroid formations of varying diameter, mainly toward the site opposite the ependyma. The latter is here formed by densely arranged cuboidal cells between which small fragments or droplets of neurosecretory substance can only rarely be seen.

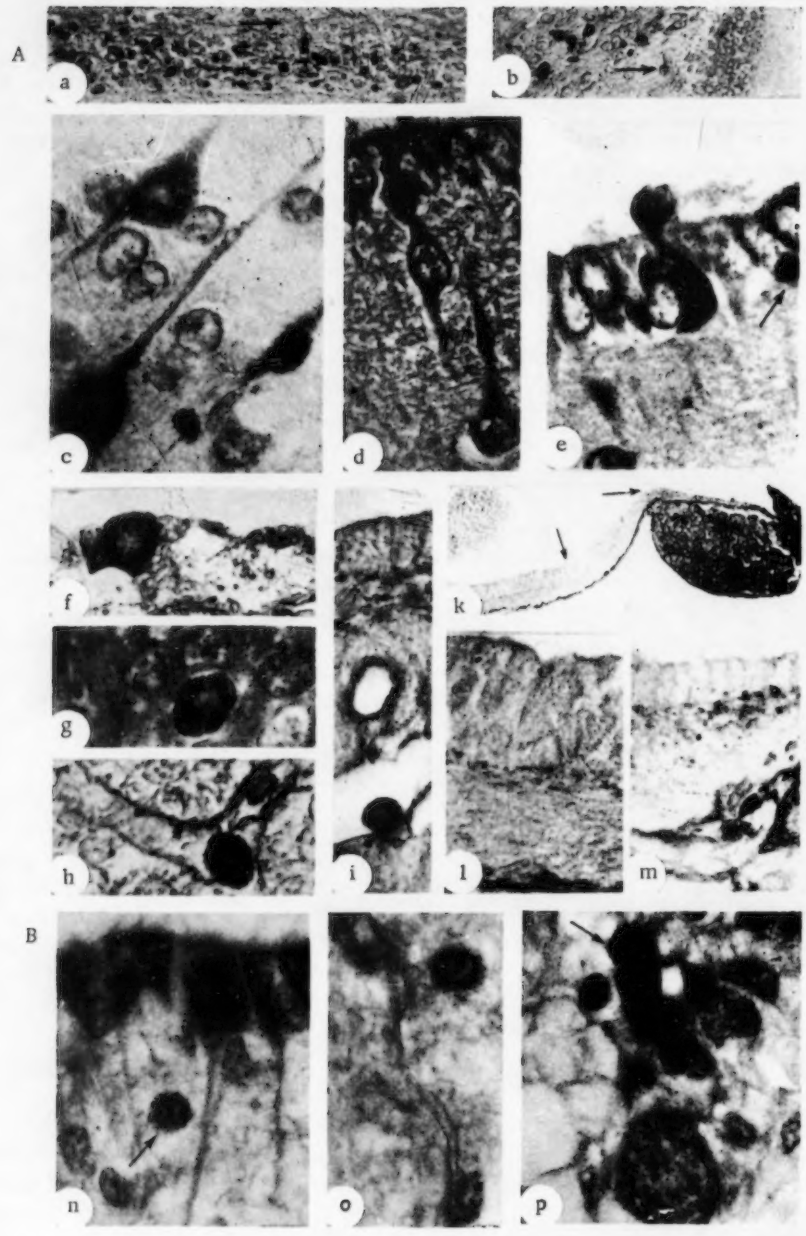
The more dorsally situated ependyma which covers the compact part of the preoptic nuclei has a quite different appearance (see Fig. c): here the cells are very tall, of typical bipolar character (see Figs. d, e). Along the processes of the ependymal cells, between the cell bodies and on the apical surface, fragments and droplets as well as dilated processes of the neurons containing a granular secretion can be seen (see Figs. d, e). In this region the neurosecretory cells are of larger size (see Fig. c). Part of the secretion which is formed by these cells enters the surrounding nervous tissue. The bulk of the neurosecretory substance, however, moves here toward the side of the ependyma (see Figs. b, d). On sections cut in different planes and stained with the aid of various methods a great number of droplets can be seen in the immediate vicinity of the ependyma. In this region we found small cells with a spheroid nucleus and a dense zone of compact cytoplasm which gave a brilliant color when stained with aldehyde-fuchsin (see Fig. e, arrow). The tinctorial properties of the cytoplasm in these cells are similar to those of the neurosecretory substance. These cells in the ependymal region do not yet display the dense deep color which is typical for the cells found in the more caudal parts of the brain. This fact suggests that the neurosecretory substance accumulates gradually in the cells. Similar cells can be found in various parts of the brain but they are not found anterior to the preoptic region. Their character resembles that of the reticulopituicytes described by Romeis [17]. They are freely wandering and can be found on the tall-celled ependyma in the region of the preoptic nuclei, on the ependyma of the IIIrd ventricle, in the infundibular region, in the capillary lumen of the brain stem and in the walls of the infundibulum between the ependymal cells of the median eminence and on its base as well as between the cells of the intermediate lobe of the pituitary gland (see Figs. f,g,h,i,k,l,m). Such cells are never found in the anterior lobe of the hypophysis; these wandering cells are not "mastcells": they are of different size and lack the characteristic granulation.

We extirpated the preoptic part of the hypothalamus in tadpoles and young yearlings of R. esculenta and the spadefoot toad Pelobates fucus. This operation was followed by a rapid reduction of the neurohypophysis. The region of the lesion and also the more caudally situated parts of the brain were investigated histologically. We did not in all operations achieve the complete resection of the preoptic fossa down to the optic chiasma or produce in all cases necrosis of the hypothalamic nuclei. Under these conditions the number of wandering cells increased considerably in the immediate vicinity of the lesion as well as in the cavity of the infundibulum; the cytoplasm of these cells contained large quantities of neurosecretory substance which gave a positive P.A.S. reaction.

The transport of the neurosecretory substance by wandering cells does not represent a phenomenon restricted only to lower vertebrates, as it occurs also in mammals. Figures n, o and p show intensively staining wandering cells in the region of the median eminence, part of which migrate toward the underlying tuberal lobe of the pituitary gland. As we said above with regard to amphibia these cells acquire a dendritic shape when entering the nervous tissue; when they are situated on the ependyma surface or within a blood vessel or in the cerebrospinal fluid, however, they are of round shape. Such cells occur in great numbers also in the proximal part of the neurohypophysis of young puppies (see Figs. o,p) and frequently swell up to take on the shape of large spheroid formations filled with fine granules.

The data discussed above show that along with nerve fibers wandering cells take part in the transport of the neurosecretory substance. It is characteristic that the latter mainly occur in the immediate vicinity of the source of neurosecretion as well as in the region where this secretion is being used up–namely the neurohypophysis. The staining reaction of these cells to polysaccharide stains corresponds to their high content of neurosecretory substance.

Other data suggest that the neurosecretory substance can be transported through the cerebrospinal fluid [8,12,13]. Collin and Barry [8] observed cells situated on the ependyma surface discharging granules of secretory sustance into the cerebrospinal fluid of the IIIrd ventricle. Discharge of the secretion on the ependyma surface and into the lumen of the preoptic cleft, not only by means of wandering cells but also in the shape of small granules or larger droplets (see Figs. d,e), could repeatedly be observed in our material. These findings as well as the difference found in the state of the ependymal lining in some parts of the cerebral cavities induced us to accept the conclusion of Löfgren [14] according to which the neurosecretory substance is transported to the recess of the infundibulum with the flow of



Various means of formation and transportation of the neurosecretory substance. A) in amphibia; B) in mammals; a, b) parasagittal section through the preoptic region of R. esculenta, at a magnification of ×240; the arrow shows the ventrodorsal direction; a) abundance of large droplets of neurosecretory substance; b) movement of the droplets of neurosecretory substance toward the ependyma of the IIIrd ventricle (staining according to Halmy-Dyban); c) neurons containing a multitude of neurosecretory granules in the dorsal part of the nucleus in a toad at a magnification of ×1200 (aldehyde-fuchsin); d, e,) tall ependymal cells in the dorsal part of the nucleus of R. temporaria (aldehyde-fuchsin); d) progression of the secretion between the ependymal cells; e) secretion reaching the surface of the ependyma, at a magnification of ×1200; f,g,h,i) wandering cells at a magnification of ×1800 (Halmy-Dyban stain); f) between the ependymal cells of the median eminence of R. esculenta; g) in the intermediate lobe of the pituitary gland of the spadefoot toad (Pelobates fucus); h) in a capillary lumen within the brain tissue of a yearling of the spadefoot toad Pelobates fucus; i) in the same animal in the vicinity of a capillary in the median eminence; k,l,m) the bottom of

cerebrospinal fluid. Here the neurosecretory substance is precipitated and enters through the loose layer of ependymal cells lining the bottom of the infundibulum the numerous capillaries of the pituitary portal system or directly the tuberal lobe; this transport is mediated by the glia cells.

And indeed in the region of the bottom of the infundibulum the glia cells are, in the animals investigated by us (amphibia and mammals), very tall and arranged loosely in two-three rows. The ependymal lining seems highest in those small areas of the deepening in the IIIrd ventricle where the outflow of neurosecretory substance into the cerebrospinal fluid is most marked of all. A similar picture of a stratified ependyma was found in amphibia also at the bottom of the infundibulum during the metamorphosis of the frog. This organization develops already in the tadpole stage. At this time the intermediate and distal lobes of the pituitary gland are formed under the influence of the earlier differentiated neurohypophysis and begin to function. Figures k, l, m show that the bottom of the infundibulum consists of a mass of longitudinally arranged non-myelinated nerve fibers which are covered by a layer of tall ependymal cells with a loose cuticular surface which shows fine processes; although the nerve fibers are evenly distributed within the bulk of the infundibular wall the granules of the neurosecretory substance are nevertheless mainly situated near the base of the ependymal cells both in the area anterior to the pituitary gland and in the region of the median eminence.

The local changes of the ependyma in two topographically separated areas deserve attention inasmuch as the glia cells represent mediators and apparently also transformers of the neurosecretory substance on its way into the blood stream. A concentration of granules and fragments of the neurosecretory substance round the endothelium of the capillaries in the median eminence of the neurohypophysis is a common finding in histological sections. The morphological organization of the pituitary gland in the larvae of amphibia deserves attention: the infundibular deepening represents the deepest point in the path of the cerebrospinal fluid; the spoon-shaped base of the infundibulum is bent in the caudal direction. It is to be expected that in such an elongated bag-shaped formation the liquid will stagnate and exert a higher pressure upon the wall of the base than in other parts of the brain. In other words it is here that the most favorable conditions for accumulation and diffusion of the neurosecretory substance into the wall of the infundibulum exist. It is in this region that considerable concentration of granules and fragments of secretion giving a positive stain reaction can be observed immediately under the ependyma. In whatever way the neurosecretory substance is transported it is subjected to changes, changes which become manifest in its tinctorial properties. On its path the neurosecretory substance passes through several (glial and endothelial) membranes.

The results of transection of the hypothalamo-hypophyseal tract quoted above seem to constitute a serious argument against the existence of additional ways of transportation of the secretion as discussed above [11]. After this operation the neurosecretory substance disappears from the neurohypophysis and accumulates at a site located proximally of the point of transection. These experiments showed with great force of conviction that an outflow takes place along the nerve fibers of the hypothalamo-hypophyseal tract. The transection leads, however, as is well known, to degenerative changes in the hypothalamic nuclei themselves, a fact which cannot but impair the other ways of transportation of the neurosecretory substance as well. Far from denying the flow of the neurosecretory substance along the axons we thus report data concerning other ways of transportation of this substance to the pituitary gland. The existence of such additional ways is further supported by the repeatedly observed discrepancy between the quantity of neurosecretory substance found in various parts of the hypothalamo-hypophyseal tract on the one hand and the two main parts of the neurohypophysis on the other hand.

SUMMARY

Neurosecretory nuclei of the hypothalamus and the area of infundibulum were subjected to histological investigation in tadpoles and frogs of various age. Different methods were used. Both intact animals and those subjected to diencephalon excision were studied. Apart from this, albino rats and puppies were also taken as objects for this

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the infundibulum of a spadefoot toad tadpole – the stage before the onset of metamorphosis (Halmy-Dyban stain); k) magnification × 144; l, m) magnification × 1200; at the base of the tall ependymal cells numerous neurosecretory granules can be seen; n) tall ependymal cells in the region of the median eminence of the neurohypophysis of a puppy; intensively stained wandering cell at a magnification of × 1800 (Gomori-hematoxylin); o,p) proximal part of the neurohypophysis of a puppy at a magnification of × 1800 (aldehyde-fuchsin); o) two cells in the vicinity of a capillary; p) a wandering cell and two spheroid accumulations of neurosecretory granules of different size.

experimental work.

The presence of neurosecretion was established not only in the bodies and the processes of the neurons and the surrounding tissues, but also in the wandering cells accumulating in their cytoplasm the positive aldehyde-fuchsinophilic substance (and in this connection staining intensively). The data discussed concerns the transmission of neurosecretion through the cerebrospinal fluid; the active state of the cells in the ependymal lining of the neurohypophysis (its proximal part) was especially emphasized.

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THE DAILY PERIODICITY OF THE MITOTIC ACTIVITY IN THE CORNEAL EPITHELIUM OF STARVING RATS AND MICE

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 52, No. 7, pp. 103-106, July, 1961
Original article submitted November 9, 1960

In the literature devoted to problems of cell multiplication numerous facts have been described which suggest the existence of a marked daily periodicity in the mitotic activity of a number of mammalian tissues [1,2,3,10]. Some papers report a relation between the daily periodicity of cell multiplication and lighting [1,7,10], or the feeding habits [1,9]. It is, however, not quite clear which of these factors is decisive in determining the character of the daily periodicity of cell multiplication. For example numerous authors have produced evidence for the influence of lighting upon the daily periodicity [1,7,10] but only a few studies were devoted to the effect of alimentary stimuli upon the daily periodicity and the conclusions of the authors were of contradictory character [1,9].

To elucidate the factors which determine the daily periodicity of cell multiplication in the corneal epithelium we carried out experiments on starving rats and mice in which the influence of the alimentary factor had been excluded for a prolonged period. Our previous experiments served as the starting point for the present study. In these [4,5] we had shown that cell multiplication in the corneal epithelium of starving animals can for a considerable time be kept at a normal level.

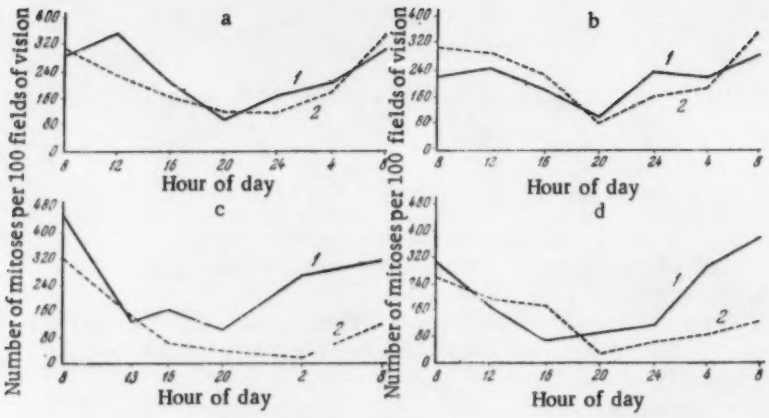
METHOD

The experiments were carried out on male white mice and white rats. The average weight of the experimental mice was 20 g. In the experiments on rats three weight groups were used: 1) heavyweight animals: 250-260 g; 2) animals of medium weight: 150-160 g; 3) animals of light weight: 50-60 g. The heavyweight rats were killed between the second and third day of starvation. This period was chosen in view of the fact that in our previous experiments no decrease in the mitotic activity had been observed in animals of that weight group after a similar period of starvation [5]. Rats of medium weight were killed on the fourth-fifth day of starvation, assuming that by that time possible changes in the mitotic activity would be found [5]. Lightweight rats were killed on the third-fourth day and mice on the first-second day of starvation: in the earlier experiments we had observed a marked decrease in the mitotic activity in lightweight rats and mice by that time [4,5]. Throughout the period of starvation the experimental animals received no food at all but were given unlimited quantities of water. The control animals received the normal quantities of food. In all other respects the conditions under which the experimental and controlanimals were kept were completely identical. The animals were killed every four hours beginning from 8 a.m. to 8 a.m. on the next day. Five experimental and five control animals were killed simultaneously. Altogether 205 white rats and 70 white mice were used for the present study.

The mitoses were counted on total preparations of the corneal epithelium after staining with hematoxylin. The various phases of the mitotic cycle were counted separately. The whole cornea was reviewed. The mitotic index was defined as the number of cells found in a state of division in a hundred fields of vision, which corresponded to 1 mm^2 area within the corneal epithelium.

RESULTS

Heavyweight rats. Experiments carried out on heavyweight rats show that the mitotic activity of the corneal



The daily periodicity in the mitotic activity of the corneal epithelium of starving rats and mice: a) heavyweight rats; b) medium-weight rats; c) lightweight rats; d) mice; 1) control animals; 2) starving animals.

epithelium underwent in both the fed animals and the starving animals considerable changes in the course of the day (see Fig. a). The maximum number of mitoses was found in the morning hours; in the course of the day the number of cells in a state of division decreased and the lowest number of mitoses was found at 8 p.m. (in both the starving and the fed rats the difference between the maximum and the minimum was found to be significant; p=0.000). Beginning from midnight the number of mitoses gradually increased and practically reached by 8 a.m. of the next day the original level. In no period could a statistically significant difference in the number of cells in a state of division be found between the control rats and the experimental rats respectively. The mean mitotic index for the day reached in the control animals 220 and in the starving animals 207 (this difference is statistically insignificant).

Rats of medium weight. In this series of experiments the daily periodicity was studied after periods of starvation ranging from 96 to 120 hours. The average values of the mitotic index are set forth in the Fig. b. The pattern of the mitotic activity in animals of this weight group was hardly different from the picture of changes described in the previous experiment. In both the starving and the fed animals the maximum number of mitoses was found in the morning hours (between 8 and 12) and the minimum activity in the eveninghours (8 p.m.). The mean daily mitotic index reached in the starving animals 244 and in the control animals 215 (the difference was statistically insignificant). It thus appears that in this weight group too we failed to find any changes in the character of the daily periodicity during starvation, changes which could have been regarded as a decrease in the average daily number of mitoses or as a disruption of the daily rhythm.

Lightweight rats. The mitotic activity was studied after periods of starvation ranging between 72 and 96 hours. The curves characterizing the mitotic activity set forth in Fig. c show that in the control animals the daily periodicity was the same as in animals of the former weight groups: the maximum number of mitoses was found in the morning hours (8 a.m), a minimum in the evening (8 p.m.).

Almost throughout the whole period of experiment a considerable decrease in the number of cells in state of division could be found in the starving animals compared with the fed animals. Besides it is a fact deserving attention that the minimum of the mitotic activity was shifted from 8 p.m. to 2 a.m. The difference in the number of mitoses between the peak and the bottom of the curve proved to be statistically significant (p=0.002).

Toward the end of the experiment (8 a.m. next day) the mitotic activity showed a slight increase. This increase was found not to be statistically significant (p=0.454). The mean daily mitotic index reached in the control rats 244 mitoses per 1 mm^2 cornea and in the starving rats 120 mitoses. This difference was found to be statistically significant (p=0.01). It thus appears that in the described series of experiments we were able to find an appreciable decrease in the number of cells in a state of division in starving animals compared with the corresponding control experiment almost throughout the day. There was, however, no complete levelling of the indices characterizing the mitotic activity, as a certain similarity to the usual rhythm of cell multiplication was preserved.

Mice. Throughout the first half of the experiment (starvation from 24 to 48 hours) no statistically significant difference in the number of mitoses was found in the starving and control animals respectively. The difference between the maximum and the minimum was found to be statistically significant (p=0.002 for the control animals and p=0.0000 for the starving animals). In the second half of the experiment beginning from 8 p.m. a general decrease in the mitotic activity could be observed in the starving animals but a statistically significant difference was found only toward the end of the experiment at 4 a.m. and 8 a.m. (p = 0.005 and p = 0.006 respectively). At the same time the starving mice preserved a tendency toward an increased number of mitoses in the morning hours. The difference between the maximum and minimum number of mitoses was characterized by a considerable degree of significance (p=0.037). The mean daily mitotic index reached in the control mice 209 and in the starving mice 135 (p = 0.013).

The results of four series of experiments quoted in the present paper concerning the daily periodicity of the mitotic activity show that a marked rhythm of cell multiplication exists not only in the control animals, which were fed, but also in the starving animals. In heavyweight and medium weight rats no statistically significant difference can be found in either the character of the curves of the daily rhythm of cell multiplication or the average daily number of mitoses compared with the control animals even after prolonged starvation (for three-five days). In both the starving and the fed animals the daily curves characterizing the mitotic activity have a peak in the morning hours (between 8 a.m. and noon) and the deepest point in the evening (8 p.m.).

In our opinion this fact can be explained with the fact that in the starving rats the content of protein in the eye, including the cornea, which represents the main plastic material, does not change compared with the corresponding control animals [8]. Consequently the presence of a certain amount of energetic reserves in the animal body makes it possible to maintain cell multiplication in the corneal epithelium of starving animals at normal levels. Only in lightweight rats and mice, which animals have a higher intensity of metabolism than heavyweight rats, could a decrease in the mitotic activity be observed. But even in these animals the daily periodicity is preserved notwithstanding the considerable fall in the average daily number of mitoses.

We were thus unable to confirm on the example of the corneal epithelium of white rats and white mice the conclusions of Blumenthal [9] obtained in experiments on guinea pigs concerning the daily rhythm of cell multiplication in starving animals.

As far as the factors determining the daily periodicity of the mitotic activity are concerned the results of a number of authors [1,7,10] must be emphasized who showed that a marked interrelation exists between artificial changes of sequence of day and night and a full reversion in the daily periodicity of the mitotic activity in the corneal epithelium as well as in the epithelium of the skin, the tongue and the esophagus. The influence of prolonged changes in the feeding habits [1] became manifest only in changes in the daily periodicity of the mitotic activity in the intestinal epithelium and the epithelium of the tongue, i. e., the tissues of those organs directly connected with the process of digestion, and did not become manifest in the mitoses found in the corneal epithelium and the epithelium of the skin.

Summing up the findings discussed in this paper and the investigations of the authors quoted above [1,7,10] we come to the conclusion that apparently it is not the feeding habits but the light factor which determines the regulation of the daily periodicity in the mitotic activity of the corneal epithelium. Light possibly acts as signal correlating the behavior reaction of the animals and thus changing the relative proportion between plastic and energetic forms of metabolism and thus determining one or the other level of cell multiplication at various times of the day.

SUMMARY

The author studied the 24 hour periodicity of mitotic activity in rats belonging to three different weight groups and mice during starvation. In heavy rats (250-260 g) and in those of moderate weight (150-160 g) starved for 2-5 days no statistically authentic differences were noted either in the 24 hour rhythm of mitosis, or in the mean 24 hour number of the latter (in comparison with control). Only in rats of low weight (50-60 g) and in mice the mitotic activity decreased considerably at the end of the experiment with prolonged starvation. However, the 24 hour rhythm of mitosis was retained to some extent. The 24 hour mitotic curves were characterized by a rise during the morning and reduction in the evening both in the animals on normal diet and in the starving ones.

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THE INFLUENCE OF BACTERIAL POLYSACCHARIDES UPON EXPERIMENTAL TISSUE REGENERATION

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 52, No. 7, pp. 107-110, July, 1961
Original article submitted July 11, 1960

In recent years studies concerning the biologically active bacterial polysaccharides were met with considerable interest, as parenteral administration of these substances increases the resistance of laboratory animals to infection and to ionizing radiation to a considerable degree. The influence of bacterial polysaccharides upon tissue regeneration is of great practical importance and requires further experimental study.

We studied the influence of two polysaccharides – cyine, prepared from Azotobacter xylinum, and a polysaccharide prepared from E. coli strain K-12 (preparation K-12).

METHOD

One of the polysaccharides was administered to white mice at the times stated below before or after an injury was inflicted on the foot of the animals under deliberately non-aseptic conditions by cutting through along about one half of its length on the lateral edge of the foot the soft tissues and the fourth and fifth metatarsal bones. Cyine was administered in a single dose of 200 μg by intraperitoneal injection, preparation K-12 in a dose of 5 μg by intravenous injection. The control animals were given injections of normal saline. Altogether 135 mice (90 experimental and 45 control mice) were used which were killed consecutively in the course of 25 days.

RESULTS

In the control mice the injury is quickly followed within 20-30 min by massive edema of the foot, the tibiotarsal joint, the greater part of the shin, and sometimes the region of the knee joint and the adjacent part of the thigh. The marked edema accompanied by intensive hyperemia of the foot persisted for 4-5 days. In the majority of control mice the region of the wound was covered during the first seven-eight days by a purulent scab surrounded by a broad zone of hyperemia. Histological and microbiological investigation revealed during the first four-five days the presence of a great number of organisms in the wound.

On the sixth-seventh day fibroblasts and the first argyrophile fibers appeared in the region of the wound among extensive infiltrates consisting of histiocytes and lymphocytes. Along the line of the operative incision the cellular detritus persisted; no epidermization could be observed (Fig. 1, a). Resorption of the superficial layer of the facing ends of the transected bones could be observed. Along these surfaces poorly differentiated connective tissue cells could be seen. On the tenth day no typical scar tissue had yet formed in the wound region (Fig. 2, a). In some parts cartilage cells appeared along the surfaces of the transected bones. On the fifteenth day a well developed layer of hyaline cartilage could be seen on the ends of the transected bones in the majority of control animals; this layer was surrounded by rather thick bundles of collagenous fibers and fibroblasts. On the 20th-25th day the bone fragments became consolidated under formation of a callus; in the central part of the callus the hyaline cartilage persisted. Even on the 25th day considerable infiltrates consisting of histiocytes and lymphocytes could be found in the scar tissue.

In the experimental animals the edema quickly disappeared within 12-24 hours if cyine was injected 2-24 hours after the operation. If it was injected 24 hours before the operation a somewhat lesser edema developed in the injured foot than in the control animals. Injection of K-12 exerted no appreciable influence upon the develop-

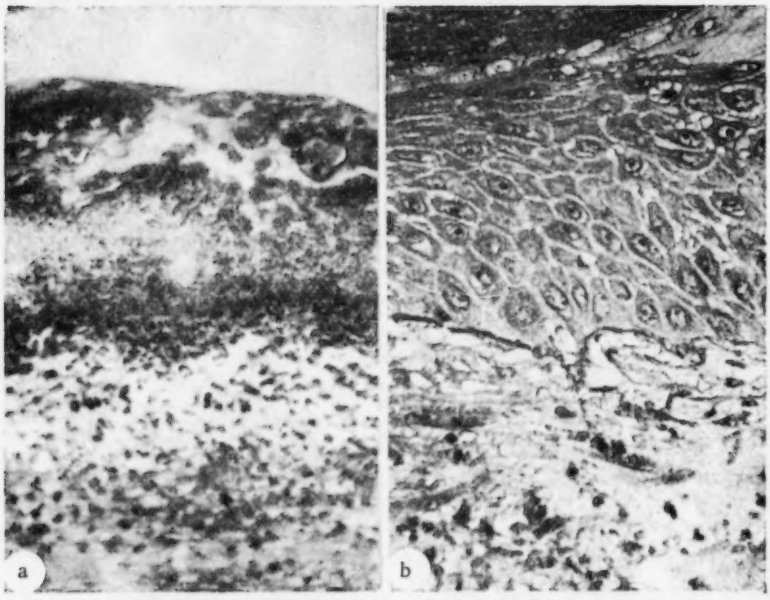

Fig. 1. State of the site of incision in a control mouse (a) and an experimental mouse (b) on the seventh day of the experiment. In the control animal a mass of cellular detritus can be seen; in the experimental animal epithelization of the cicatrizing wound can be seen. Staining with iron-hematoxylin and aniline blue. Magnification × 300.

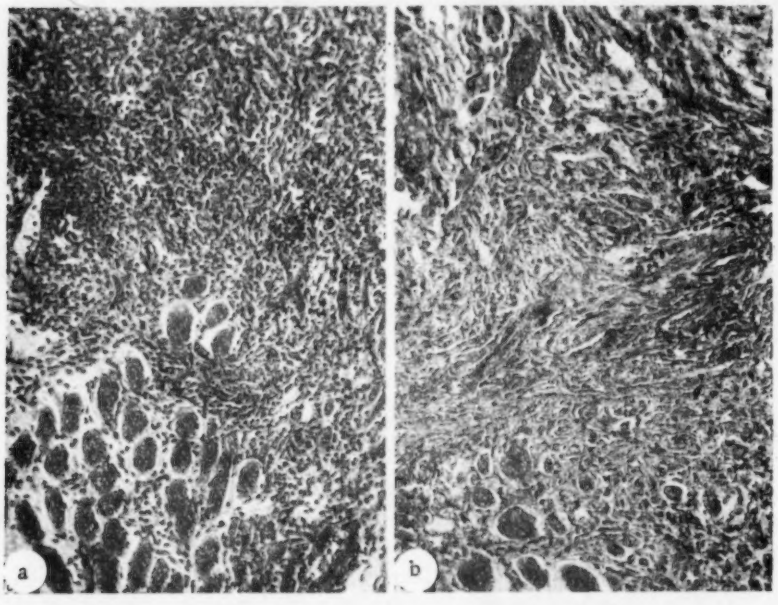

Fig. 2. The state of the wound in a control mouse (a) and an experimental mouse (b) on the tenth day of the experiment. In the control animal an intensive inflammatory reaction, in the experimental animal, the presence of scar tissue can be seen. Van Gieson stain, magnification × 160.

ment of edema. Microscopical investigation of preparations and selective cultures revealed that the wounds of animals treated with either cyine or preparation K-12 before the operation or within the first day after the operation were practically free of microorganisms. If one of the two polysaccharides was administered before the operation or in the first days after the injury the inflammatory infiltrate was less extensive.

On the seventh day of the experiment a considerable number of newly formed collagenous fibers could be seen in seven out of ten mice which had been given injections of cyine and four out of nine mice which had been given injections of the preparation K-12. The greatest part of the incision was covered by regenerating epidermis; a basal membrane appeared along its internal border (Fig. 1, b). Ten days after the operation almost no residue of necrotic muscle fibers could be seen in the region of the wound in five out of 11 mice treated with cyine and three out of nine mice treated with the preparation K-12; the formation of scar tissue could be observed (Fig. 2, b) and along the surface of the transected bones a broad zone of hyaline cartilage could be seen. On the 20th day bone tissue developed on the ends of the transected bones in five out of eight mice treated with cyine and three out of seven mice treated with the preparation K-12; the clefts in the bone were filled with elements of the red bone marrow, a phenomenon which in the control animals could be observed only on the 25th day. In mice killed on the tenth day of the experiment the scars were as a rule much narrower than in the control mice.

Administration of cyine or preparation K-12 was never followed by a deterioration in the state of the wound or delayed regeneration. Consequently the unfavorable influence upon the regeneration and the state of the tissues reported by some authors [3,4,5] must not be regarded as characteristic for all polysaccharides, in particular not for those which stimulate resistance to infection. Above we remarked that following the administration of cyine and the preparation K-12 the wound regeneration took place in a considerable part of the experimental animals more rapidly than in the control animals. At the same time we had shown earlier that a single cyine injection has no influence upon the speed of encapsulation of foreign bodies. In consequence the more rapid regeneration found on the model of traumatic inflammation can hardly be regarded as the consequence of the direct influence of polysaccharides upon those elements which secure the proliferative processes. It seems that in the case of traumatic inflammation the more rapid regeneration is enhanced (among the factors studied so far) by the very rapid liquidation of the wound infection and the more rapid lysis of the necrotic tissues due to the activation of the phagocytic elements [1], and improved blood supply in the wound region due to the absence of edema [2], which latter causes compression of small veins.

SUMMARY

There was no unfavorable effect noted from parenteral administration of polysaccharides obtained from Az. xylinum (cyine) or from E. Coli K-12 (preparation K-12) on the tissue regeneration in foot trauma inflicted on mice. In a number of cases tissue regeneration in animals which received cyine or K-12 preparation was even quicker than in control animals. Evidently, this results from the complex of favorable shifts, such as a more rapid elimination of the infective agents, activation of macrophagic elements and removal of the factor of venous compression by edema fluid.

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PECULIARITIES IN THE REGENERATION OF THE HEMATOPOIETIC TISSUE IN CHICK EMBRYOS EXPOSED TO EXTERNAL γ-RADIATION

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 52, No. 7, pp. 110-114, July, 1961
Original article submitted July 5, 1960

The lesions of the hematopoietic organs developing under the influence of ionizing radiation are regarded as a hypoplastic or aplastic state of the hematopoietic organs [4-9,12]. After the initial stimulation of the bone marrow, the more rapid maturation of the hematopoietic elements and their entering into the peripheral blood stream a profound inhibition of the hematopoietic function develops. In severe cases panmyelophthisis-an irreversible condition of the bone marrow involving complete cessation of hematopoiesis-develops. In cases of myeloparesis regenerative processes start at a certain stage of radiation sickness which lead to a more or less complete restoration of the disrupted function.

Radiation sickness is followed by disorders of both blood cell proliferation and blood cell differentiation. The latter becomes particularly manifest in the restoration period, in the course of which the regeneration may adopt a number of pathological features. Here we have in mind the occurrence of elements characteristic for the embryonal megaloblastic erythropoiesis and of leukemoid conditions.

When speaking of the development of radiation lesions in embryos it must above all be emphasized that although the processes which take place in the hematopoietic organs of embryos exposed to radiation are in principle similar to the same processes in adult animals exposed to radiation, the specific features characterizing embryonal hematopoiesis and the high regenerative capacity of these tissues bring about a number of essential changes.

The literature contains only a few reports concerning the influence of ionizing radiation upon embryonal hematopoiesis [1-3,10,11,13,14]. A. A. Zavarzin and co-workers emphasized that the blood cells of an early chick embryo are very sensitive and that regeneration takes place very rapidly [3]. Others reported a general decrease in the number of circulating elements [13] and an inhibition of blood cell maturation [14]. A general inhibition of hematopoiesis and distorted regeneration accompanied by signs of megaloblastic hematopoiesis have also been reported with regard to mammalian embryos [1,10,11].

There are however no systematic observations concerning the regenerative processes in embryos during the early stages of development. The present paper is devoted to this question.

METHOD

Chick embryos on the fifth day of incubation were exposed to γ-rays emitted by Co^{60} (capacity 1.45-1.2 r per min, dose 1000 r). In the period studied by us (5-12th day of incubation) the only hematopoietic organ present in the embryo consists of the network of hematopoietic capillaries in the yolksac; here the erythropoiesis takes place within the blood vessels and the leukopoiesis in the intervascular reticular tissue. Under normal conditions the products of leukopoiesis appear in the peripheral blood only toward the end of the period studied by us and in small numbers. We investigated sections taken from the walls of the yolksac (fixation in Zenker-formol, staining with iron hematoxylin, according to Yasvoin and with azur-eosin) and films made from the peripheral blood.

RESULTS

If one studies the hematopoietic organs of embryos exposed to radiation the most striking fact is the rapidity with which profound disorders develop in the hematopoiesis. Immediately after exposure to radiation changes can be observed in the original stem cells (Stammzelle) which at that period constitute just as in the control animals the overwhelming majority of cellular elements in the hematopoietic capillary. These cells possess also under normal conditions ameboid mobility. For that reason they frequently show short pseudopodia in the preparations. After exposure to radiation the configuration of the cells undergoes certain changes. Elongated thick protoplasmatic processes of irregular shape develop. In some places they lose their connection with the cell body and form freely lying fragments which give an intensive basophilic stain. The nuclei are of irregular shape and show deep indentations; frequently the constriction is deep enough to divide the nucleus into fragments. It can be observed that the damaged cells are taken up by phagocytic reticulo-endothelial elements. The endothelial layer of the capillaries is thickened and loosened; in some areas perivascular edema can be observed.

Fig. 1. Section through the yolksac wall of a six-day-old embryo exposed to radiation. Perivascular edema. The hematopoietic capillaries are empty. Hematoxylin stain, objective 20×, apochromatic, eye-piece 7×.

On the day following the exposure to radiation (sixth day of incubation) the inhibition of hematopoiesis progresses vehemently. In a number of cases a picture of complete aplasia can be observed in the network of hematopoietic capillaries (Fig. 1). The hematopoietic elements disappear completely (or almost completely). Round the blood vessels massive perivascular edema develops. In the zone of edema the reticular elements appear compressed. Their nuclei are in a state of pycnosis and karyorrhexis.

In a lesser number of embryos the destruction of young hematopoietic elements is not as complete as that described above. Here too however the hematopoietic areas are diminished and contain degenerative forms.

A more or less marked perivascular edema is visible in almost all sections prepared from the wall of the yolksac of 7-, 8-, 9-day-old embryos. On the subsequent days edema is found less frequently and is less intensive; even on the 12th day of incubation, however, edema may occur, even in preparations in which the regeneration of hematopoietic areas is well marked. Although the extensive perivascular edema delays the regenerative process to a certain degree in view of the fact that part of the mesenchymal hematopoietic reserves is destroyed, this does not prevent the onset of this process almost immediately after the end of the exposure to radiation.

In the undamaged cells of the reticular syncytium mitoses become more frequent. The endothelial cells take on a round shape and protrude into the lumen of the hematopoietic capillary. The whole course of the subsequent restoration, however, is characterized by disorders in the differentiation and maturation of the blood cells. On sections prepared from the wall of the yolksac of a 7-8-day-old embryo (2nd and 3rd day after exposure to radiation), less frequently at later stages, an increase in the number of reticular stroma cells and an increased frequency of mitoses in these cells can be observed. The total number of early basophilic hematopoietic elements, however, remains very low and they often sustain degenerative changes in the shape of cytoplasmic vacuolization, as well as karyorrhexis. One of the sections prepared from the hematopoietic organs of an 8-day-old embryo is particularly interesting from this point of view: in this case the hyperplasia of the reticuloendothelial apparatus reached a considerable degree. The perivascular spaces were filled with a great number of reticular cells. In the lumen of the

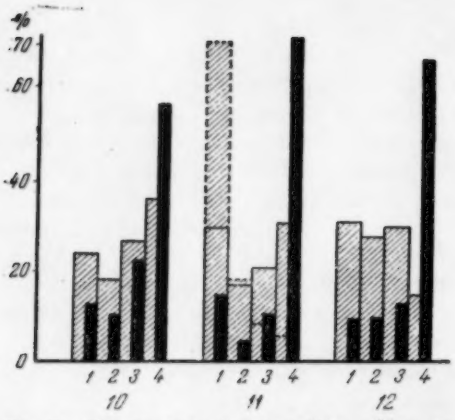

Fig. 2. The percentual composition of hematopoietic cells in a hematopoietic capillary. Black columns: control; hatched columns: after radiation. On the abscissa the stages of maturation, on the ordinate the days of incubation are plotted.

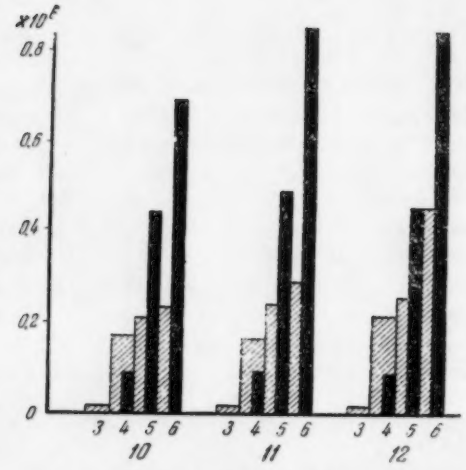

Fig. 3. The number of cells of the erythroid series in 1 mm^3 of peripheral blood. Key to figure same as in Fig. 2.

hematopoietic capillary numerous atypical cells of irregular, elongated shape and with an unevenly basophilic protoplasm could be seen. Many of these cells had protoplasmatic processes with the aid of which part of the cells preserved their connection with the capillary endothelium. No normal hematopoietic cells could be seen. These facts show that the regeneration of the hematopoietic elements encounters some difficulties, notwithstanding the increased proliferation of the reticuloendothelial cells; in some cases the reticuloendothelium produces atypical cells instead of the normal hematopoietic elements.

On the subsequent days the development of the process suggests a permanent inhibition of differentiation, at first in the early basophilic stages, later at the stage of basophilic and polychromatophilic erythroblasts (Fig. 2). Fig. 2 was constructed following the principle of partial myelograms. As it is impossible to draw a perfect analogy between the blood cells of a chick embryo and the generally accepted hematological nomenclature the consecutive stages of erythroblast maturation are referred to by numbers: stages 1-3 correspond to the stages between the hemocytoblast and the basophilic erythroblast; stage 4 corresponds to the polychromatophilic erythroblast. It is quite apparent that 5-7 days after the exposure to radiation the hematopoietic elements of the earlier stages predominate in the hematopoietic capillaries. In one of the embryos exposed to radiation the most immature blood cells constituted 70% of all hematopoietic elements found in the hematopoietic capillaries (columns indicated by a dotted line on Fig. 2). Here, the mitotic activity (3% of the cells were found to be in a stage of mitosis) corresponded to the mitotic activity found in the control sections. It can consequently be assumed that in this case there was no increased proliferation of the cells but an inhibition of differentiation and of the transition into the later stages. This led to an accumulation of undifferentiated immature cells.

In our previous paper [2] we had shown by analyzing the composition of the peripheral blood that exposure of embryos to radiation on the 5th and 8th day of incubation leads, as a rule, to a return to the ontogenetically earlier megaloblastic erythropoiesis. Indeed, elements characteristic for megaloblastic erythropoiesis could be seen in the hematopoietic elements in the shape of large cells containing considerable quantities of hemoglobin, but preserving the structure of the nucleus typical for earlier basophilic elements: a well marked large nucleolus accompanied by a hardly visible chromatin network within the nucleus.

The primitive leucocytes which are formed in the perivascular spaces can be found in unchanged appearance almost in all sections prepared from the hematopoietic organ of the embryos exposed to radiation, even in those cases in which the erythropoiesis suffered considerable damage. This fact is possibly connected with the relatively low activity of leucopoiesis in chick embryos at early stages of embryogenesis.

The peculiarities of hematopoiesis in chick embryos consist particularly in the fact that at early stages of development the hematopoiesis takes place both in the hematopoietic organ and in the peripheral blood. On the

5th and 6th day of incubation the hematopoietic capillaries contain mainly poorly differentiated cells. Cells of the intermediate stages, which have the highest mitotic activity, are mainly found in the peripheral blood; here their maturation is completed. Later the exodus of immature elements into the blood becomes more intensive, but even on the 12th day of incubation the peripheral blood contains 6-7% of polychromatophilic erythroblasts.

Fig. 3 shows the quantitative proportions of various stages of red cell maturation in 1 mm^3 peripheral blood. It can be seen that the number of polychromatophilic and mature red cells (stages 5-6) is much lower than in the control sections, whereas the absolute number of red cells is increased. This fact also suggests an inhibition of red cell maturation with a consequent increase in the number of immature elements.

Radiation damage in chick embryos is thus characterized by an extremely rapid depletion of the hematopoietic areas and the development of perivascular edema, by an early reaction of the reticuloendothelium, inhibition of blood cell maturation and disorders of regeneration, accompanied by the appearance of elements typical for megaloblastic hematopoiesis.

SUMMARY

Chick embryos underwent gamma-irradiation on the 5th day of incubation. Destruction of the endothelium and massive perivascular edema occur in the hematopoietic capillaries of the yolk-sac within the very first hours after irradiation. The hematopoietic elements disappear almost completely. Already on the next day, parallel with the continuing destruction, there appear regeneration elements in the form of proliferation of the reticuloendothelium and formation of new hematopoietic elements. Inhibition of the differentiation processes often leads to the accumulation of elements with low differentiation and prevents the restoration of normal blood composition. Marked affection of hematopoiesis leads in a number of cases to the reestablishment of megaloblastic hematopoiesis.

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THE BLOOD INDICES OF THE HEALTHY GUINEA PIG

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 52, No. 7,
pp. 115-118, July, 1961
Original article submitted September 14, 1960

The guinea pig is a convenient animal to study. However, up till now there has been a disagreement between the different reports of the mean values of its blood indices [1,4-8].

We have therefore set out to determine their normal mean values.

METHOD

A study was made of the blood of 100 guinea pigs of both sexes whose weight ranged from 400 to 720 g. The following quantities were determined: hemoglobin; number of erythrocytes, leucocytes, reticulocytes and thrombocytes, and erythrocyte sedimentation rate. Counts were also made of the relative and absolute number of the blood cells. The results were treated statistically.

RESULTS

The values obtained are shown in Table 1.

TABLE 1. Indices for Healthy Guinea Pig Blood

Index	$M \pm m$	$\pm\sigma$	Normal range $M \pm 1\sigma$
Hemoglobin, %	85±0.76	7.64	74.4—92.6
Erythrocytes (millions)	5.15±0.06	0.57	4.58—5.72
Reticulocytes, %	12.8±0.62	6.25	6.55—19.05
Thrombocytes (thousands)	298.35±0.1	72.0	226.3—370.3
Erythrocyte sedimentation rate	2.1±0.16	1.15	1.0—3.2
Leucocytes (thousands)	14.584±0.48	4.78	9.8—19.3
Basophils:			
%	0.4±0.08	0.59	0—1.0
Absolute number	79.5±15.8	112.0	0—191.5
Eosinophils:			
%	3.2±0.54	3.8	0—7.0
Absolute number	700±133.0	944	0—1 644
Neutrophils with rod-shaped nuclei:			
%	0.3±0.06	0.42	0—0.7
Absolute number	81.6±14.2	99.8	0—181.4
Polymorphonuclear neutrophils:			
%	29.7±2.69	19.05	10.65—48.75
Absolute number	5 951.6±658.0	4 660	1 291.6—10 611.6
Lymphocytes:			
%	63.0±2.82	20.0	43.0—83.0
Absolute number	10 426.1±666.0	4 720.0	5 706.1—15 146.1
Monocytes:			
%	3.7±0.33	2.36	1.3—6.1
Absolute number	636.2±64.4	456.0	180.2—1 092.2

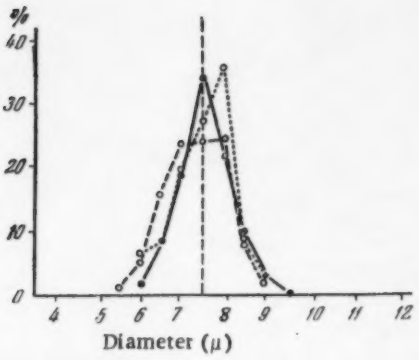

Fig. 1. Price-Jones curves for the healthy guinea pig.

Mean red cell diameter was measured with an AM-9-2 eyepiece, to an accuracy of 0.1μ. The average of studies on 50 animals was 7.50 ± 0.025μ, with a normal range of variation (M± 1σ) from 7.33 to 7.69μ. Price-Jones curves for the healthy guinea pig are given in Fig. 1.

Bone marrow smears were made from the iliac crest of 40 animals. In each case, 400 cells were measured, and the mean value found. The results were treated statistically (Table 2).

In 1957, I. I. Gitel'zon and I. A. Terskov [2] proposed the method of acid fragility tests, which enabled a morphologically homogeneous mass of erythrocytes to be divided according to their stability, and allowed their age and condition to be determined.

We have made fragility tests on 50 animals *. A 0.004 N solution of HCl was used as the hemolytic agent. The following

TABLE 2. Myelogram of the Healthy Guinea Pig

Type of cell	$M \pm m$	$\pm\sigma$	Normal range M ± 1σ
Reticulo-endothelial cells	1.41±0.170	1.08	0.33—2.49
Hemocytoblasts	0.95±0.129	0.82	0.03—1.77
Promyelocytes	0.76±0.098	0.62	0.14—1.38
Neutrophil myelocytes	3.16±0.166	1.05	2.11—4.21
Eosinophil myelocytes	1.0±0.118	0.75	0.25—1.75
Neutrophil metamyelocytes	5.55±0.162	1.03	4.52—6.58
Eosinophil metamyelocytes	0.70±0.102	0.65	0.05—1.35
Neutrophils with rod-shaped nuclei	16.06±0.822	5.20	10.86—21.26
Eosinophils with rod-shaped nuclei	3.66±0.176	1.11	2.55—4.77
Polymorphonuclear neutrophils	23.33±0.623	3.94	19.39—27.27
Polymorphonuclear eosinophils	1.22±0.135	0.85	0.37—2.07
Basophils	0.48±0.090	0.57	0—1.05
Monocytes	3.16±0.350	2.25	0.91—5.41
Lymphocytes	16.88±0.790	4.98	11.9—21.86
Plasma cells	0.89±0.110	0.74	0.15—1.63
Megacaryocytes	1.12±0.032	0.19	0—0.31
Proerythroblasts	0.29±0.038	0.24	0.05—0.53
Macroblasts	1.43±0.181	1.15	0.28—2.58
Normoblasts	17.88±0.876	5.54	12.34—23.42
Broken cells	0.95±0.330	1.97	0—2.92

indices were found: duration of hemolysis: 7-8 min (mean value 7 ½ min); greatest number of cells lyzed after 2.5 min , and occasionally after 2 min.

After the results had been treated statistically, they were plotted as shown in Fig. 2.

The agranulocytes of the guinea pig contained inclusions which were first described in 1888 by the Tomsk therapeutist M. G. Kurlov, and which are known as Kurlov bodies. Various figures have been given for the number of these inclusions. M. G. Kurlov himself thought that the bodies were present in 15-20% of the cells, Shilling reported 1-8%, Laione — 5%, and Schermer gives figures from 0 to 4%.

Our studies have shown that the bodies are present in 1-10% of the cells (and on average in 5-7%).

We have found no account of the nucleic acids in the cells of the circulating guinea pig blood. Our studies were made on 25 normal guinea pigs, and the nucleic acid content of the blood cells was determined by the method of Brachet and Feulgen.

We found that the ribonucleic acid (RNA) is contained in the cytoplasm of the monocytes and lymphocytes as small dark red granules. The cytoplasm of the monocytes is very rich in RNA, which is diffusely distributed, and frequently obscures the nucleus. The lymphocytes may be divided into two groups, according to their RNA content.

* This part of the work was carried out together with O. S. Golosov.

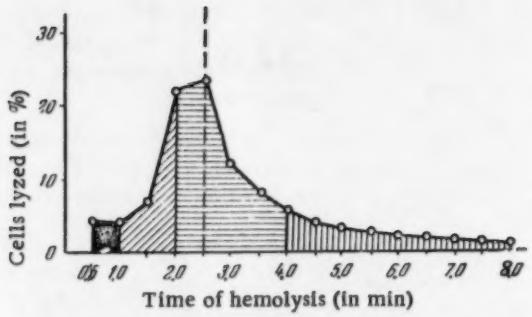

Fig. 2. Curve of acid hemolysis, obtained by statistical treatment of 50 cases. Different kinds of shading distinguish the groups according to their degree of stability.

The first group comprises lymphocytes having a thick band of cytoplasm. In them, the RNA is distributed throughout the whole of the cytoplasm, but is concentrated chiefly in two zones, one at the edge of the cell and the other round the nuclear membrane. The second and larger group is composed of cells with a large nucleus and a narrow band of cytoplasm. In these lymphocytes the RNA forms a narrow band round the nucleus. The nuclei of all the blood cells are rich in DNA; the nucleoli of the lymphocytes contain RNA.

In this account we have attempted to determine more completely the blood indices of the guinea pig.

SUMMARY

Statistical results on the blood indices of healthy guinea pigs are given. For the first time a distribution curve has been obtained which shows the stability of healthy guinea pig erythrocytes to lysis in acid solution; the measurements were made by the method of Gitel'zon and Terskov.

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A NEW METHOD FOR PRODUCING AN ISOLATED GASTRIC POUCH

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 52, No. 7,
pp. 119-120, July, 1961
Original article submitted May 12, 1960

The formation of an isolated gastric pouch is a method which makes possible an objective study of gastric secretion, and enables some information on motor function to be obtained. The qualification is necessary, because the separation of the pouch either partially or completely from the main stomach appreciably alters the motility of the latter. The problem is still more severe if two gastric pouches are formed simultaneously. It is then almost impossible to determine the motility of the main portion of the stomach. In our opinion, the motor responses made in stomachs with isolated pouches should be interpreted as the reaction of muscular tissue in general to various stimuli.

Many of the operations described [1,2,3,9,10,11,12,14,15,16] have the common feature that the cavity of the pouch is formed by cutting through all the layers of the stomach, while the isthmus is made from the serous and muscular wall, which is not cut. In the method proposed by A. V. Solov'ev [9,10], bilateral innervation is preserved, so that a detailed study of secretion can be made, and some information on motility may be obtained.

We here describe a modification of the method of forming an isolated pouch which can be applied to any part of the stomach. The innervation and blood supply remain intact, and there is scarcely any change in the anatomical relationships. The operation is performed as follows (Figs. 1 and 2). An incision is made through all the layers of the anterior wall of the stomach, parallel to the greater curvature. The anterior gastric wall is folded outward where it is intended to form the isolated pouch. In the middle of this region, the normal intestinal fistula is established. Around the fistula, the mucosa is cut in a circle having a diameter of approximately 5 cm. The edges of the mucosa are pulled back 1 cm in both directions. The edges of the inner portion are then sewn up with a purse-string suture and are stretched as an arch over the fistula. In this way, the isolated pouch is formed from all three layers of the main stomach, while the arch is formed only from the stretched mucosa.

a b

Fig. 1. Initial and final stages of the operation. a) Incision in the anterior gastric wall (the vessels to the greater and lesser curvatures are compressed by clamps); b) external view of stomach after formation of the gastric pouch.

To increase the strength of the arch, the mucosa is sewn from above in a cross-shaped suture so as to include the submucosal layer of the isthmus. The suture makes for better isolation and accelerates the growth of connective tissue which effectively separates off the gastric pouch which has been formed. The free edges of the mucosa may readily be drawn together with a purse-string suture to complete the lining of the main stomach. The incision in the anterior wall of the stomach is sewn up in the usual way, so that the main stomach remains intact.

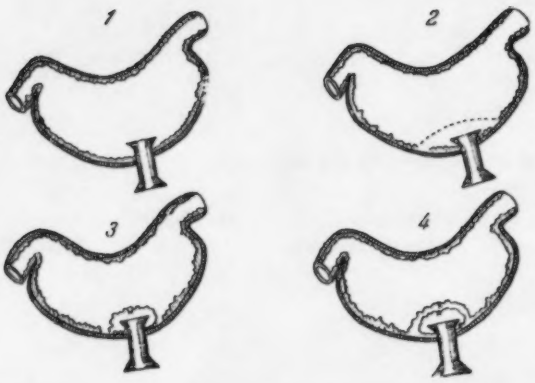

Fig. 2. Principal stages of the operation (diagrammatic). 1) A fistula is established in the stomach in the usual way; 2) the mucosa is freed around the fistula; 3) an isolated gastric pouch is formed by drawing the mucosa together over the fistula; 4) by means of a purse-string suture the gap in the mucosa of the main stomach is closed, which makes the lining continuous.

The operation is comparatively simple and takes 30-40 min. The secretion obtained shows that the innervation and the blood supply are well preserved. If a small balloon is placed in the fistula, or if the fistula is merely connected by means of a rubber tube to a Marie's capsule, it is easy to obtain a record of the movements of the main stomach which are not distorted on account of numerous incisions in the gastric wall. The operation is very convenient when it is required to make a simultaneous study of secretion and motility.

SUMMARY

A new method is described of forming an isolated gastric pouch in any part of the stomach. Its advantages are that the innervation and the blood supply remain intact and anatomical relationships remain almost unchanged. A stomach pouch formed by this method is convenient for making a simultaneous study of secretion and motility. The operation is illustrated in Figures 1 and 2.

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TESTING SMALL INSULIN CONCENTRATIONS BY MEANS OF ISOLATED EPIDIDYMAL RAT FAT

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(Presented by Active Member AMN SSSR V. G. Baranov)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 52, No. 7,
pp. 121-124, July, 1961
Original article submitted March 17, 1960

In recent years, methods have been proposed for testing small insulin concentrations by means of their action on fatty tissue. The basis of the method is that insulin increases the utilization of glucose, its oxidation to carbon dioxide, the formation of fat from glucose, etc. The effect of insulin on glucose utilization by fatty tissue has not been sufficiently studied, and the different laboratories concerned have usually used quite a small range of insulin concentrations. The results from different laboratories, and even from those using similar methods of its action on epididymal rat fat are widely divergent: in one case the minimum concentration of insulin was 1 micro-unit per ml [2], in another it was 10 micro-units [6], in a third 100 micro-units per ml [11]. The maximal effect has been reported to occur in one case at a concentration of 100 micro-units per ml [2], and in another at 10,000 micro-units per ml [11]. The differences may to some extent depend on the strain of rat used, and on their nutrition [7]. A relevant factor may also be the use in many cases of gelatine to prevent absorption of insulin on the walls of the glass vessel [5]. However, these considerations cannot explain the whole of the divergence in results, which are probably to be attributed to other differences in technique.

METHOD

The experiments were carried out on white male rats of various weights, fed on a free choice of diet. They were killed by decapitation, and the epididymal fat was quickly removed. It was important to avoid damage to the fatty tissue, and to reduce to a minimum the time for which it was cooled in the air. In our experiments, not more than 2-3 min elapsed between removing the fat and placing it in the incubator. After it had been removed, it was weighed on a torsion balance and placed in a 25 ml Erlenmeyer flask containing the incubation medium. The fatty tissue was weighed before and after incubation, and in many cases it was found to increase by 10-60%, and the amount of water taken up was not proportional to the weight of tissue. We have therefore thought it incorrect to determine the weight of the tissue after incubation, and in all our experiments the calculations were made in terms of the freshly removed fat.

After the fat had been placed in the flask, the latter was closed with a rubber cork containing tubes through which a gaseous mixture was passed for $1\frac{1}{2}$ -2 min. It was found from many experiments that a suitable composition for the gaseous mixture was 5% carbon dioxide, while the remaining 95% of the mixture could be either air or oxygen. When the air was replaced by oxygen, no change in the results was found. Similar results for the formation of carbon dioxide by fatty tissue were obtained whether the mixture contained air or oxygen [4]. On this account we used a mixture consisting of 5% carbon dioxide and 95% air. After aeration, the tubes were closed, and the flask was placed in a Warburg apparatus. Incubation at 37° was continued for 3 hours, and the flask was shaken at a rate of 120 strokes per minute.

The absorption of glucose by epididymal rat fat, and the effect on it of insulin vary greatly according to the animal used, and for this reason, in order to obtain reliable results, it is important to incubate tissue from several animals simultaneously in a single flask, or else to repeat the experiment many times. On account of the wide variations in the sensitivity of the fat of different animals to incubation, it is justifiable to incubate the fat from several animals at the same time in a single flask, only if the amount of fat contributed by each animal is the same

and this condition is not easily attained without causing trauma to the fatty tissue. It is therefore preferable to use fat from a single animal, and to repeat the determination several times. For this reason we took two portions of epididymal fat from each animal, one from each side. One was incubated in a medium with, and the other in a medium without insulin. It should be noted that different portions of the fat of a single animal may show different sensitivities [1].

The experiments were made on 48 portions of fat weighing 100-700 mg, and it was found that for an insulin concentration of 1,000 micro-units per ml the absorption of glucose was proportional to the weight of the tissue, the co-efficient of proportionality being 0.72. The absorption was greatly reduced when the weight of the sample exceeded 300 mg, and in subsequent experiments samples of such a size were not used. In many cases it was found that the sensitivity of the deeper portions of epididymal fat was less than the more peripheral parts'. Similar results were obtained by determining the formation of carbon dioxide from glucose due to epididymal fat [4]. We therefore used two weighed portions of peripheral epididymal fat from each animal.

The incubation medium was that of Krebs and Henseleit [8], which contained 400 mg% of glucose and 200 mg% of gelatine. Previous experiments have shown that when gelatine is added the effect is considerably enhanced.

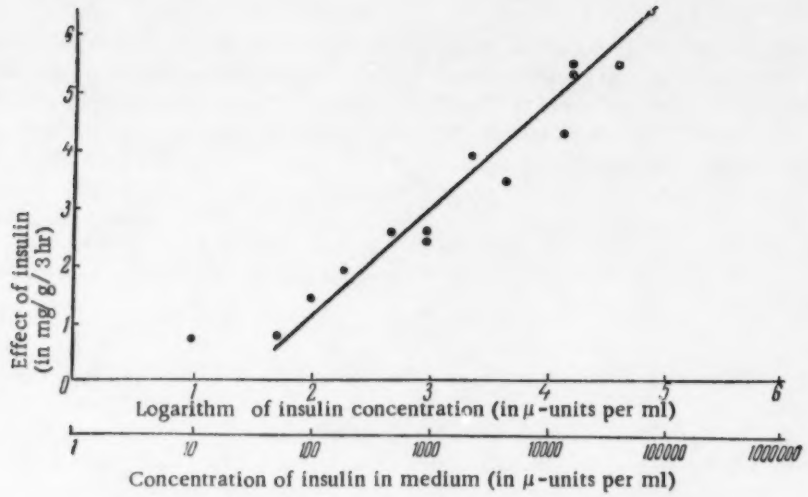
The insulin was supplied by the Leningrad Meat Plant; it was series No. 39, the activity was 24 units per mg, it was not free from glucagon, and it was recrystallized once. A stock solution of insulin was made in 0.01 N hydrochloric acid, and it had an activity of 50 units per ml; subsequently, portions were diluted to the required concentration.

The experiments were carried out simultaneously on 5 animals. The absorption of glucose by fatty tissue was calculated from the excess glucose remaining in the medium after incubation, and it was expressed as mg of glucose absorbed per g of fat per 3 hours' incubation. The effect of insulin was measured by the difference in the amount of glucose absorbed in a medium with or without insulin, and was expressed as mg of glucose per g of fat per 3 hours. The glucose concentration of the medium was determined by the method of Somodzhi and Nelson [9,10], and by the protein precipitation method of Frank and Kirberger [3].

Because the experiment was carried out on 5 animals, the correlation between insulin concentration and its effect was obtained in terms of the mean value for the group, and not from the separate determinations. In fact, by this method, the co-efficient of correlation shows how accurately it is possible to determine insulin concentration in the medium from its mean effect on 5 animals. Of course, it would be possible to obtain greater accuracy with a greater number of rats, but two Warburg apparatuses would then be required, which is inconvenient.

Effect of Various Insulin Concentrations on the Absorption of Glucose by Weighed Portions of Epididymal Rat Fat

Insulin concentration in medium (in micro-units per ml)	Log. of insulin concentration	Number of animals	Effect of insulin (in mg of glucose/g of tissue/3 hr)	
			Results of different determinations	Mean value
10	1.0	5	0; 0.71; 0.76; 0.84; 1.23	0.71
50	1.7	5	0; 0.48; 0.54; 0.55; 2.16	0.75
100	2.0	4	0.70; 2.45; 2.90; 3.73;	2.44
100	2.0	5	0.33; 0.79; 1.26; 2.26; 2.43	1.41
200	2.3	5	0.55; 1.67; 2.06; 2.45; 2.56	1.84
500	2.7	5	0.28; 1.98; 2.25; 3.03; 4.97	2.50
1 000	3.0	5	1.40; 2.15; 2.37; 2.85; 3.05	2.36
1 000	3.0	5	0.97; 2.31; 2.49; 2.95; 3.86	2.50
2 500	3.4	5	1.36; 1.83; 3.88; 5.57; 6.36	3.80
5 000	3.7	5	0.05; 3.01; 3.55; 4.71; 5.62	3.38
15 000	4.2	5	1.90; 2.50; 2.75; 6.30; 7.68	4.22
20 000	4.3	5	3.64; 4.41; 5.18; 5.92; 7.45	5.32
20 000	4.3	5	2.94; 4.94; 5.42; 5.46; 7.40	5.23
50 000	4.7	5	3.62; 3.92; 5.18; 5.90; 8.13	5.35
100 000	5.0	5	3.08; 5.32; 6.68; 7.37; 9.25	6.34
500 000	5.7	4	2.60; 5.28; 5.90; 9.78	5.89



The effect of insulin at different concentrations. The points represent mean values for 4-5 determinations. The regression line has been drawn.

In determining the correlation between insulin concentration and its effect, we used the well-known method of expressing not the actual concentration, but the logarithm to the base ten of the concentration in micro-units of insulin per ml of medium. The effect was calculated from the difference in the amount of glucose utilized in media with and without insulin.

In some cases, the glucose absorption by the fatty tissue was so great that the figures were not included in those from which the mean values were calculated. For example, in one of the experiments, the action of insulin in a concentration of 100 micro-units per ml was 11 mg/g/3 hours, while in another, at a concentration of 500,000 micro-units per ml it attained the value of 37 mg/g/3 hours. We were unable to find the reason for this very high sensitivity. Other authors have observed the same effect.

RESULTS

The table shows the separate and mean values of 78 determinations made in 16 experiments using 13 different insulin concentrations.

An increase in the insulin concentration of the medium above 100,000 micro-units per ml did not increase its effect, while reducing it to 5 micro-units per ml led to variable and uncertain results.

It can be seen from the table that over a range of concentrations of 50 to 100,000 micro-units per ml there is a linear relationship between the logarithm of the insulin concentration and its effect.

It could be shown statistically that for concentrations between 5 and 100,000 micro-units per ml, the co-efficient of correlation between the log. of the insulin concentration and the effect on 4-5 animals was + 0.889. For this number of experiments [14], the mean error of the co-efficient was 0.277, and therefore the co-efficient itself was 3.2 times greater than its mean error, which shows that the correlation was highly significant. The regression line was determined from the formula: $x=0.568\,y + 1.382$, where x is the logarithm of the concentration of insulin in the medium, and y is the effect of insulin in mg of glucose per gram of tissue per 3 hours.

For the concentration limits given above, the mean error of the logarithm of the concentration, calculated from the formula, is 0.25, which corresponds to a mean error in the determination of the insulin concentration of 1.78 (in certain experiments it ranged from 1.07 to 5.9; then, in 12 experiments out of the 14, the error did not exceed 2.7). For comparison, the mean error for the determination of insulin using an isolated rat diaphragm is usually taken to be 3 (i.e., the value found may be three times less or three times greater than the true amount).

Therefore, by using the regression equation, the method of determining insulin concentration in terms of the utilization of glucose by epididymal rat fat enables unknown insulin concentrations of a wide range to be determined

with sufficient accuracy.

The co-efficient of correlation which we obtained is higher than that reported elsewhere. Possibly, some reasons for such a high degree of correspondence are as follows. The correlation was determined from the mean values of several experiments carried out in parallel, which is a more precise method than using results of separate experiments, which always give a fairly wide scatter. The calculation was made not from the absolute absorption of glucose from the medium by insulin, but from the effect of the action of insulin, i. e., from the extra amount of glucose absorbed from a medium containing insulin, as compared with one free from it. We did not incubate weighed portions of tissues from several different animals simultaneously in one flask, because such a method not only does not reduce the influence of individual variations, but may actually increase it. Other unknown factors may also play an important part, and they may include properties of the insulin itself, the strain of rats, their nutrition, etc.

SUMMARY

The study of glucose uptake by rat epididymal fat has demonstrated that the effect occurs at concentrations above 10μ units per ml; it then increases progressively up to a concentration of 100,000μ units per ml. Within a range of concentration from 50 to 100,000μ units per ml, there is a linear relationship between the logarithm of the insulin concentrations in the medium and its effect; the correlation co-efficient is + 0.889. A regression equation was calculated which gave a mean error for the determination of the insulin concentration of 1.78. Thus, this method is not only simple, but is also sufficiently precise for the determination of insulin concentrations in an incubation medium.

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ABBREVIATIONS MOST FREQUENTLY ENCOUNTERED IN RUSSIAN BIO-SCIENCES LITERATURE

Abbreviation (Transliterated)	Significance
AMN SSSR	Academy of Medical Sciences, USSR
AN SSSR	Academy of Sciences, USSR
BIN	Biological Institute, Botanical Institute
FTI	Institute of Physiotherapy
GONTI	State United Sci-Tech Press
GOST	All Union State Standard
GRRRI	State Roentgenology, Radiology, and Cancer Institute
GTTI	State Technical and Theoretical Literature Press
GU	State University
I Kh N	Scientific Research Institute of Surgical Neuropathology
IL (IIL)	Foreign Literature Press
IONKh	Inst. Gen. and Inorganic Chemistry (N. S. Kurnakov)
IP	Soil Science Inst. (Acad. Sci. USSR)
ISN (izd. Sov. Nauk)	Soviet Science Press
Izd.	Press
LEM	Laboratory for experimental morphogenesis
LENDVI	Leningrad Inst. of Dermatology and Venereology
LEO	Laboratory of Experimental Zoology
LIKhT	Leningrad Surgical Institute for Tuberculosis and Bone and Joint Diseases
LIPZ	Leningrad Inst. for Study of Occupational Diseases
LIPK	Leningrad Blood Transfusion Institute
Medgiz	State Medical Literature Press
MOPISh	Moscow Society of Apiculture and Sericulture
MVI	Moscow Veterinary Institute
MZdrav	Ministry of Health
MZI	Moscow Zootechnical Institute
LOKhO	Leningrad Society of Orthopedic Surgeons
NIIZ	Scientific Research Institute of Zoology
NINKhI	Scientific Research Institute of Neurosurgery
NIU	Scientific Institute for Fertilizers
NIUIF	Scientific Research Institute of Fertilizers and Insecticides
NIVI	Veterinary Scientific Research Institute
ONTI	United Sci. Tech. Press
OTI	Division of Technical Information
RBO	Russian Botanical Society
ROP	Russian Society of Pathologists
SANIIRI	Central Asia Scientific Research Institute of Irrigation
SANIISh	Central Asia Scientific Research Institute of Sericulture
TsNII	All-Union Central Scientific Research Institute
TsNTL	Central Scientific and Technical Laboratory
VASKhNIL	All-Union Academy of Agricultural Sciences
VIG	All-Union Institute of Helminthology
VIEM	All-Union Institute of Experimental Medicine
VIR	All-Union Institute of Plant Cultivation
VIUAA	All-Union Institute of Fertilizers, Soil Science, and Agricultural Engineering
VIZR	All-Union Institute of Medical and Pharmaceutical Herbs
VNIRO	All-Union Scientific Institute of Fishing and Oceanography
ZIN	Zoological Inst. (Acad. Sci. USSR)

Note: Abbreviations not on this list and not explained in the translation have been transliterated, no further information about their significance being available to us. - Publisher.

Soviet Journals Available in Cover-to-Cover Translation

ABBREVIATION	RUSSIAN TITLE	TITLE OF TRANSLATION	PUBLISHER	TRANSLATION BEGAN Vol.	Issue	Year
AÉ	Atomnaya énergiya	Soviet Journal of Atomic Energy	Consultants Bureau	1	1	1956
Akust. zh.	Akusticheskii zhurnal	Soviet Physics – Acoustics	American Institute of Physics	1	1	1955
	Antibiotiki	Antibiotics	Consultants Bureau	4	1	1959
Astr(on). zh(urn).	Astronomicheskii zhurnal	Soviet Astronomy–AJ	American Institute of Physics	34	1	1957
Avto(mat). svarka	Avtomaticheskaya svarka	Automatic Welding	British Welding Research Association (London)		1	1959
	Avtomatika i Telemekhanika	Automation and Remote Control	Instrument Society of America	27	1	1956
	Biofizika	Biophysics	National Institutes of Health*		1	1957
	Biokhimiya	Biochemistry	Consultants Bureau	21	1	1956
Byull. éksp(erim). biol. i med.	Byulleten' éksperimental'noi biologii i meditsiny	Bulletin of Experimental Biology and Medicine	Consultants Bureau	41	1	1959
DAN (SSSR) / Dokl(ady) AN SSSR	Doklady Akademii Nauk SSSR	The translation of this journal is published in sections, as follows:				
		Doklady Biochemistry Section	American Institute of Biological Sciences	106	1	1956
		Doklady Biological Sciences Sections (Includes: Anatomy, biophysics, cytology, ecology, embryology, endocrinology, evolutionary morphology, genetics, histology, hydrobiology microbiology, morphology, parasitology, physiology, zoology sections)	American Institute of Biological Sciences	112	1	1957
		Doklady Botanical Sciences Sections (Includes: Botany, phytopathology, plant anatomy, plant ecology, plant embryology, plant physiology, plant morphology sections)	American Institute of Biological Sciences	112	1	1957
		Proceedings of the Academy of Sciences of the USSR, Section: Chemical Technology	Consultants Bureau	106	1	1956
		Proceedings of the Academy of Sciences of the USSR, Section: Chemistry	Consultants Bureau	106	1	1956
		Proceedings of the Academy of Sciences of the USSR, Section: Physical Chemistry	Consultants Bureau	112	1	1957
		Doklady Earth Sciences Sections (Includes: Geochemistry, geology, geophysics, hydrogeology, mineralogy, paleontology, petrography, permafrost sections)	American Geological Institute	124	1	1959
		Proceedings of the Academy of Sciences of the USSR, Section: Geochemistry	Consultants Bureau	106-123	1 6	1957- 1958
		Proceedings of the Academy of Sciences of the USSR, Sections: Geology	Consultants Bureau	106-123	1 6	1957- 1958
		Doklady Soviet Mathematics	The American Mathematics Society	131	1	1961
		Soviet Physics–Doklady (Includes: Aerodynamics, astronomy, crystallography, cybernetics and control theory, electrical engineering, energetics, fluid mechanics, heat engineering, hydraulics, mathematical physics, mechanics, physics, technical physics, theory of elasticity sections)	American Institute of Physics	106	1	1956
		Proceedings of the Academy of Sciences of the USSR, Applied Physics Sections (does not include mathematical physics or physics sections)	Consultants Bureau	106-117	1	1956-1957
Derevoobrabat. prom-st'.	Derevoobrabatyvayushchaya promyshlennost'	Wood Processing Industry	Timber Development Association (London)		9	1959
	Élecktrosvyaz	Telecommunications	Massachusetts Institute of Technology*		1	1957
Éntom(ol). oboz(renie)	Éntomologicheskoe obozrenie	Entomological Review	American Institute of Biological Sciences	38	1	1959
Farmakol. (i) toksikol(ogiya)	Farmakologiya i toksikologiya	Pharmacology and Toxicology	Consultants Bureau	20	1	1957
FMM	Fizika metallov i metallovedenie	Physics of Metals and Metallography	Acta Metallurgica*	5	1	1957
Fiziol. zhurn. SSSR (im. Sechenova)	Fiziologicheskii zhurnal im. I. M. Sechenova	Sechenov Physiological Journal USSR	National Institutes of Health*		1	1957
Fiziol(ogiya) rast.	Fiziologiya rastenii	Plant Physiology	American Institute of Biological Sciences	4	1	1957
	Geokhimiya	Geochemistry	The Geochemical Society		1	1958
FTT	Fizika tverdogo tela	Soviet Physics–Solid State	American Institute of Physics	1	1	1959
Izmerit. tekh(nika)	Izmeritel'naya tekhnika	Measurement Techniques	Instrument Society of America		1	1959
Izv. AN SSSR, O(td). Kh(im). N(auk)	Izvestiya Akademii Nauk SSSR: Otdelenie khimicheskikh nauk	Bulletin of the Academy of Sciences of the USSR: Division of Chemical Sciences	Consultants Bureau		1	1952

continued

Izv. AN SSSR, O(td). T(ekhn). N(auk): Met(all). i top.	(see Met. i top.)				
Izv. AN SSSR Ser. fiz(ich).	Izvestiya Akademii Nauk SSSR: Seriya fizicheskaya	Bulletin of the Academy of Sciences of the USSR: Physical Series	Columbia Technical Translations	1	1954
Izv. AN SSSR Ser. geofiz.	Izvestiya Akademii Nauk SSSR: Seriya geofizicheskaya	Bulletin (Izvestiya) of the Academy of Sciences USSR: Geophysics Series	American Geophysical Union	1	1954
Izv. AN SSSR Ser. geol.	Izvestiya Akademii Nauk SSSR: Seriya geologicheskaya	Izvestiya of the Academy of Sciences of the USSR: Geologic Series	American Geological Institute	1	1958
Kauch. i rez.	Kauchuk i rezina	Soviet Rubber Technology	Research Association of British Rubber Manufacturers	18	1 1959
	Kinetika i kataliz	Kinetics and Catalysis	Consultants Bureau	1	3 1960
	Koks i khimiya	Coke and Chemistry USSR	Coal Tar Research Association (Leeds, England)		1 1958
Kolloidn. zh(urn).	Kolloidnyi zhurnal	Colloid Journal	Consultants Bureau	14	1 1952
	Kristallografiya	Soviet Physics – Crystallography	American Institute of Physics	2	1 1957
Metalov. i term. obrabot. metal.	Metallovedenie i termicheskaya obrabotka metallov	Metal Science and Heat Treatment of Metals	Acta Metallurgica	6	1 1958
	Metallurg	Metallurgist	Acta Metallurgica		1 1957
Met. i top.	Metallurgiya i topliva	Russian Metallurgy and Fuels	Eagle Technical Publications		1 1960
Mikrobiol.	Mikrobiologiya	Microbiology	American Institute of Biological Sciences	26	1 1957
OS	Optika i spektroskopiya	Optics and Spectroscopy	American Institute of Physics		6 1959
	Pochvovedenie	Soviet Soil Science	American Institute of Biological Sciences		1 1958
	Priborostroenie	Instrument Construction	British Scientific Instrument Research Association		1 1959
Pribory i tekhn. éks(perimenta)	Pribory i tekhnika éksperimenta	Instruments and Experimental Techniques	Instrument Society of America		1 1957
Prikl. matem. i mekh.	Prikladnaya matematika i mekhanika	Applied Mathematics and Mechanics	American Society of Mechanical Engineers		1 1958
PTÉ	(see Pribory i tekhn. éks.)				
	Problemy Severa	Problems of the North	National Research Council of Canada		
Radiotekh.	Radiotekhnika	Radio Engineering	Massachusetts Institute of Technology*	12	1 1957
Radiotekh. i élektronika	Radiotekhnika i élektronika	Radio Engineering and Electronics	Massachusetts Institute of Technology*	2	1 1957
	Stanki i instrument	Machines and Tooling	Production Engineering Research Assoc.		1 1959
	Stal'	Stal (In English)	Iron and Steel Institute		1 1959
Stek. i keram.	Steklo i keramika	Glass and Ceramics	Consultants Bureau	13	1 1956
Svaroch. proiz-vo	Svarochnoe proizvodstvo	Welding Production	British Welding Research Association		4 1959
Teor. veroyat. i prim.	Teoriya veroyatnostei i ee primenenie	Theory of Probability and Its Applications	Society for Industrial and Applied Mathematics		1 1956
Tsvet. Metally	Tsvetnye metally	Nonferrous Metals	Primary Sources		1 1960
UFN	Uspekhi fizicheskikh Nauk	Soviet Physics – Uspekhi (partial translation)	American Institute of Physics	66	1 1958
UKh	Uspekhi khimii	Russian Chemical Reviews	The Chemical Society (London)		1 1960
UMN	Uspekhi matematicheskikh nauk	Russian Mathematical Surveys	London Mathematical Society	15	1 1960
Usp. fiz. nauk	(see UFN)				
Usp. khim(ii)	(see UKh)				
Usp. matem. nauk	(see UMN)				
Usp. sovr. biol.	Uspekhi sovremennoi biologii	Russian Review of Biology	Oliver and Boyd		48 1959
Vest. mashinostroeniya	Vestnik mashinostroeniya	Russian Engineering Journal	Production Engineering Research Assoc.		4 1959
Vop. gem. i per. krovi	Voprosy gematologii i perelivaniya krovi	Problems of Hematology and Blood Transfusion	National Institutes of Health*		1 1957
Vop. onk.	Voprosy onkologii	Problems of Oncology	National Institutes of Health*		1 1957
Vop. virusol.	Voprosy virusologii	Problems of Virology	National Institutes of Health*		1 1957
Zav(odsk). lab(oratoriya)	Zavodskaya laboratoriya	Industrial Laboratory	Instrument Society of America	25	1 1959
ZhAKh Zh. anal(it). khimii	Zhurnal analiticheskoi khimii	Journal of Analytical Chemistry USSR	Consultants Bureau	7	1 1952
ZhETF Zh. éksperim. i teor. fiz.	Zhurnal éksperimental'noi i teoreticheskoi fiziki	Soviet Physics–JETP	American Institute of Physics	28	1 1955
ZhFKh Zh. fiz. khimii	Zhurnal fizicheskoi khimii	Russian Journal of Physical Chemistry	The Chemical Society (London)		7 1959
ZhMÉI Zh(urn). mikrobiol. épidemiol. i immunobiol.	Zhurnal mikrobiologii, épidemiologii i immunobiologii	Journal of Microbiology, Epidemiology and Immunobiology	National Institutes of Health*		1 1957
ZhNKh Zh(urn). neorgan(ich). khim(ii)	Zhurnal neorganicheskoi khimii	The Russian Journal of Inorganic Chemistry	The Chemical Society (London)		1 1959
ZhOKh Zh(urn). obshch(ei) khimii	Zhurnal obshchei khimii	Journal of General Chemistry USSR	Consultants Bureau	19	1 1949
ZhPKh Zh(urn). prikl. khimii	Zhurnal prikladnoi khimii	Journal of Applied Chemistry USSR	Consultants Bureau	23	1 1950
ZhSKh Zh(urn). strukt. khimii	Zhurnal strukturnoi khimii	Journal of Structural Chemistry	Consultants Bureau	1	1 1960
ZhTF Zh(urn). tekhn. fiz.	Zhurnal teknicheskoi fiziki	Soviet Physics–Technical Physics	American Institute of Physics	26	1 1956
Zh(urn). vyssh. nervn. deyat. (im. Pavlova)	Zhurnal vysshei nervnoi deyatel'nosti (im. I. P. Pavlova)	Pavlov Journal of Higher Nervous Activity	National Institutes of Health*		1 1958

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CONTENTS

Introduction
Modern Views on Neuroses
  Protective Inhibition
A Brief Outline of the History of the Method of Sleep Therapy
The Indications for Sleep Therapy in Neuroses
The Investigation of Sleep by the Method of Actography
Sleep Therapy in the Neuroses
  Composition of the Patients
  Method of Treatment
  Preparation for Treatment
  Forms of Therapeutic Sleep
  Program for the Day
  The Environment and Its Importance
  Physical Hypnogenic Factors
  The Use of Hypnotic Drugs
  The Use of Inert Substances (Hypnotic Substitutes) as Conditioned Reflex Stimuli
  The Use of Hypnosis and Suggestion
  The Daily Duration of Sleep and Its Characteristics
  The Length of the Course of Treatment
  The Changeover from Sleep Therapy to the Ordinary Routine
  Subsequent Period of Treatment
  The Results of Treatment
  Follow-up
Conclusion
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CONTENTS

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The Treatment of Coronary Insufficiency by Intraarterial Procaine Infusions

Intraarterial Procaine Infusions in the Treatment of Infectious Polyarthritis

Indications for and Contraindications to the Use of Intraarterial Procaine Infusions in the Clinical Treatment of Internal Diseases

Conclusion

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